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No. 1

CAPILLARY PERMEABILITY TO HORSE PROTEINS IN BURN-SHOCK¹

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Inasmuch as alteration of capillary permeability to plasma protein has been considered as one of the possible primary changes in shock, its quantitative measurement is important. Most of the data on permeability in shock has been derived from the use of completely foreign substances, such as dyes, or indirectly, by studies of changes in blood, lymph, or edema fluid. As far as we are aware, there has been no direct proof that capillary permeability to plasma proteins is increased in shock although alterations have usually been assumed to occur. The decrease in effective blood volume, which is universally accepted as characteristic of shock, might be accounted for by dilatation of vessels and stagnation of blood as well as by increased leakage of plasma from capillaries.

The details of a direct and quantitative method for measuring the rate of passage of a protein across the capillary endothelium of the dog are presented here. The method is a modification of that used by Field and Drinker (1) to demonstrate the passage of horse protein into lymph in normal animals. Horse serum was injected intravenously, and cervical and thoracic duct lymph were examined to determine the time and rate of appearance of the antigenically detectable serum protein. Since the horse serum was introduced by vein, any of it appearing in the lymphatics must have passed across the capillary endothelium. Therefore, direct passage of protein is measured by this technic. The method does not fulfill the requirements of a strict definition of permeability, and the factors involved in this will be considered in the discussion. The results obtained give evidence of prompt alteration of capillary permeability in both burned and non-burned areas.

METHODS. The dogs used weighed from 10 to 20 kgm. and were fed on table scraps. In most cases they were starved for approximately 24 hours before the experiment. The anesthesia used was that recommended by Wiggers (2); 3 mgm. per kgm. of morphine sulfate subcutaneously, followed in one-half hour

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by two-thirds the calculated dose of sodium barbital (175 mgm. per kgm.) given slowly by vein. During the burn this anesthesia was re-inforced briefly with ether.

The cervical and thoracic ducts were exposed by separate incisions. The cervical duct was either brought out through a skin incision and the lymph allowed to drip into a tube, or it was cannulated. The first method removed the difficulty of clotting in the cannula when the flow was slow. Thoracic lymph was secured by means of a cannula in the thoracic duct. Lymph was collected in graduated glass tubes, allowed to clot, and was later rimmed and centrifuged. To increase the flow of cervical lymph the head was either made to nod by the method of McCarrell (3) or was massaged.

The "standard burn". Dogs, anesthetized as described above, were clipped free of all hair below the axillae. Ether was administered for 2 minutes prior to the burn, and a sufficient amount was given during the burn to keep the animal quiet. In the control experiments ether was administered for 5 minutes to approximate the amount given the burned animal. Horse serum in concentration of 7.0 to 8.0 grams per cent protein was then injected intravenously, the volume being 2.0 ml. per kgm. Immediately thereafter the body was immersed up to the axillae in water at 72°C. for 60 seconds. Animals subjected to this burn, if untreated, died in from 4 to 19 hours (Parkins, 4).

Observations were continued for 3 hours after the injection of the horse serum. Lymph was collected from the two ducts, blood samples withdrawn at intervals, and the pulse, respiration and rectal temperature taken as a check on the condition of the animal. The blood was taken from the saphenous, external jugular, or femoral veins. The total protein in plasma and lymph was determined by the biuret method of Kingsley (5). Horse serum protein concentrations in plasma and lymph were determined by precipitation with anti-horse rabbit serum and the concentration of precipitate read turbidimetrically. Hematocrit determinations were done by the method of Sanford and Magath (6).

Turbidimetric method. Anti-horse rabbit sera were pooled, lyophilized, and used as a constant source. To a test tube containing 0.6 ml. of a freshly prepared standard concentration of anti-serum was added 0.3 ml. of centrifuged plasma or lymph. Each tube contained 0.1 ml. of Heller and Paul's (7) oxalate mixture to prevent clotting. The contents were thoroughly mixed, incubated at 38°C. for one hour, refrigerated overnight and centrifuged. Each precipitate was washed twice with cold physiological salt solution to remove extraneous color. Centrifuging was done in iced cups, and care was taken throughout the entire procedure to maintain a low solubility by keeping the precipitate cold. The precipitate was made up to 1.0 cc. with cold 0.85 per cent sodium chloride, mixed thoroughly, and read immediately in the Klett-Summerson colorimeter using a 420 m μ filter. Samples of plasma and lymph taken before the horse serum injection always showed some non-specific precipitation, and these values were used as the zero for the particular experiment. In a control experiment in which the animal was burned without receiving horse serum, there were no changes in the amount of this precipitate in samples of plasma and lymph taken before and for

3 hours after the burn. A calibration curve was determined by precipitating horse serum added in known concentration to dog plasma or lymph. This gave a curve relating the galvanometer deflection by the precipitate suspension to milligrams of horse-serum protein.

RESULTS. 1. *Effect of the "standard" burn on capillary permeability.* In studying capillary permeability, lymphs from the cervical and left thoracic ducts have been compared. The cervical duct drains the head and neck on that side, whereas the left thoracic duct drains the visceral area and most of the body below its point of entrance into the jugular or subclavian vein. With the type of burn used, the cervical drainage area is remote from the site of the burn, but the left thoracic duct receives fluid from the burned area.

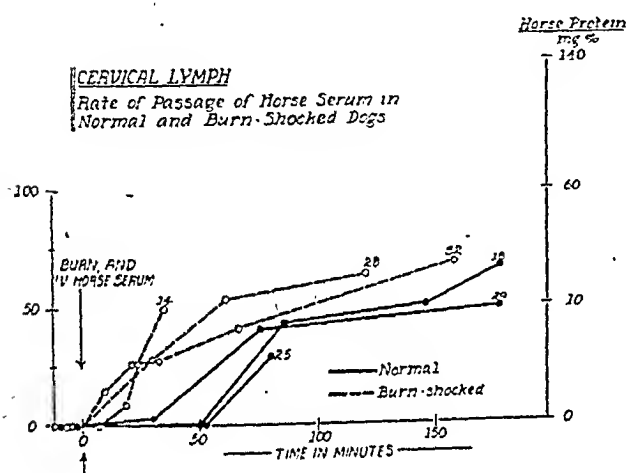


Fig. 1

Fig. 1. Chart showing the rate of passage of horse serum in normal and burn-shocked dogs for cervical lymph. Points are plotted at the end of the collection period. Numbers refer to the number of the experiment.

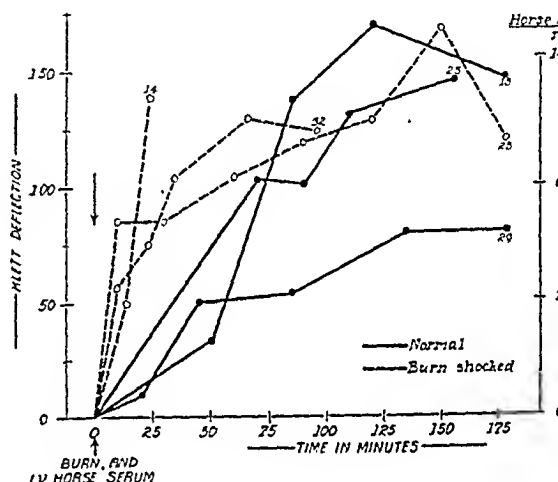


Fig. 2

Fig. 2. Chart as in figure 1, for thoracic duct lymph.

In the normal animal, comparison of the time of appearance and rate of passage of horse protein in the two areas gives a contrast between a visceral and a somatic area. The solid lines in figure 1 and figure 2 represent the data obtained with 3 normal animals. The charts show the rate of passage of horse serum across capillaries of the cervical and thoracic drainage areas. A comparison of the rates supports the suggestions made by Starling (8) on the basis of difference in lymph protein concentrations, that the thoracic drainage area (mainly visceral) is more permeable than the cervical drainage area (mainly somatic). This is shown first by the fact that whereas horse serum was detected in thoracic lymph in about 20 minutes, between 50 and 100 minutes elapsed before its appearance in cervical lymph. The latter confirms the finding of Field and Drinker (1). Second, it is seen that initially the rate of passage was greater in thoracic lymph. Third, horse serum protein concentration reached higher levels in thoracic than in cervical lymph. The concentrations reached were of the order of 10 mgm. per cent in cervical lymph, and 100 mgm. per cent in thoracic lymph.

When an animal was subjected to the "standard" burn, the shape of the curve was altered in both areas (see dotted lines in figs. 1 and 2). The horse serum was detected in the thoracic lymph in high concentration within 10 minutes. The high initial rate of appearance is readily apparent from the charts. Horse protein was detected in the cervical lymph within 10 to 20 minutes as contrasted with between 50 and 100 minutes in the normal dog. Here, too, the initial rate of passage was greater.

2. *Effect of the "standard" burn on total protein in plasma, cervical and thoracic duct lymph.* Intravenous injection of horse serum as used here did not alter

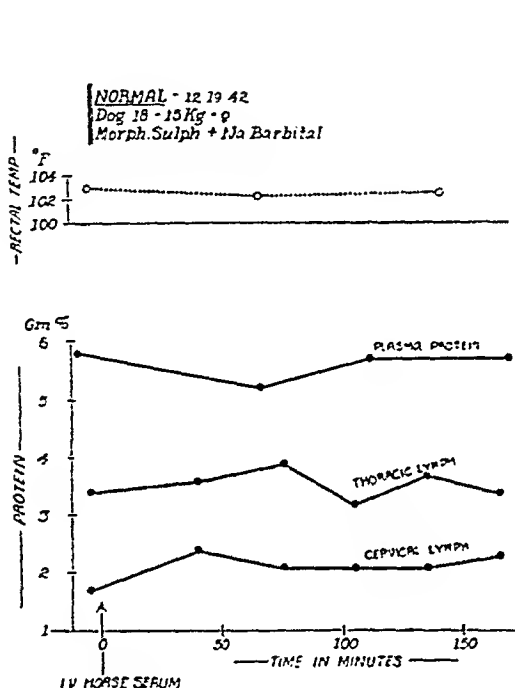


Fig. 3

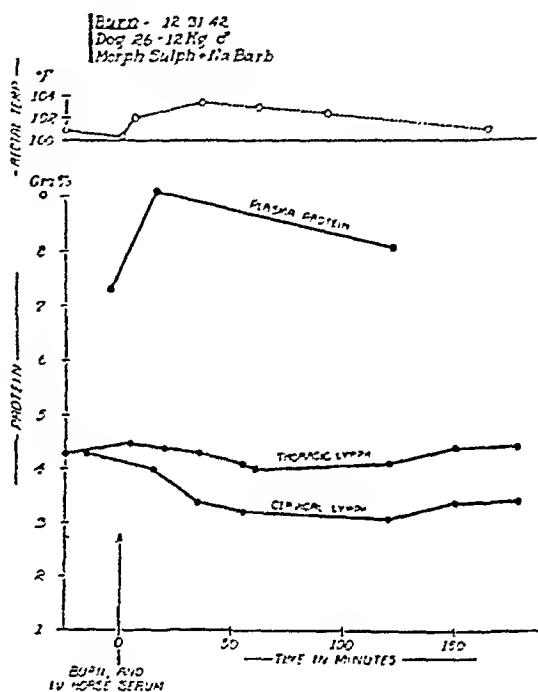


Fig. 4

Fig. 3. Chart showing protein concentrations in plasma, cervical and thoracic duct lymph over the three hour observation period following injection of horse serum. Dog 18, 15 kgm. Control animal.

Fig. 4. Chart as in figure 3 for animal subjected to "standard" burn. Dog 26, 12 kgm.

the plasma protein level of the normal dog. This is shown in figure 3, which gives results typical of 3 experiments. Over a period of 3 hours, administration of horse serum caused no significant change in the total protein concentration of either cervical or thoracic duct lymph. A typical experiment is shown in figure 3 which gives data for plasma protein, cervical and thoracic duct lymph protein. Horse serum protein does appear in lymph as shown above, but the concentrations in both blood and lymph are within the limits of error of the method for total protein determination.

Plasma protein levels rise after the standard burn as shown in figure 4. The presence of hemolysis in the serum of burned animals introduces an error in these determinations, tending to give higher than the true values. Values as

given are uncorrected. Examination of the protein concentration of thoracic duct lymph showed only small changes (fig. 4). Promptly after the burn, the concentration tended to fall and only after one or two hours did it slowly rise. The slow rise was probably due to the presence of hemoglobin. These results are surprising in view of Field and Drinker's (1) finding that heating of a limb promptly and markedly increased the protein concentration of lymph from that area (Glenn, Peterson and Drinker, 9). These workers used a burn of 100°C. for 2 minutes, which may account for the difference. Another possible explanation is that the increased rate of filtration of fluid from the large burned area more than offsets any increase in amount of protein, vascular or extravascular. Cervical lymph duct protein concentration showed a similar downward trend in our experiments, and tended to rise slowly after 2 or 3 hours. The concentration of hemoglobin here was too small to alter the colorimetric readings of protein.

3. *Other effects of the "standard" burn.* A striking degree of hemolysis in plasma was seen promptly following a burn (see among others Freeman and Schecter (10) and Glenn, Peterson and Drinker, 9). Samples of blood taken within 10 minutes after immersion showed marked hemolysis which was present throughout the three hour observation period. Hemoglobin was present in thoracic duct lymph in visible traces within 15 minutes, and by the end of 2 hours the lymph was bright red. Hemoglobin appeared in cervical duct lymph in about 35 minutes and the concentration was much lower at all times. At the end of 3 hours, it was perceptible as a faint coloring rather than the scarlet red of thoracic lymph at this time. This confirms the finding with horse protein of the difference in permeability of the cervical and thoracic drainage areas.

Hematocrit. It is well known that burns produce hemoconcentration. This was usually prompt following the standard burn, and levels of around 60 to 70 per cent were maintained during the course of the experiment.

Rectal temperature. Initial rises from the normal of 101 to 102°F. to levels around 104°F. occurred, but usually by the second hour the temperature was falling slowly (fig. 4). In some cases, the drop was followed by a secondary rise.

Lymph flow. The volume of thoracic lymph flow depends mainly on the state of the respiration and intestinal activity following the burn (Drinker and Yoffey, 11). It is therefore questionable whether much can be learned from variations in flow. Cervical lymph flow did not alter as much as thoracic flow, but the changes were in the same direction of an initial increase which slowly tapered off to the previous level.

DISCUSSION. The strict definition of permeability requires that there be measured the units of mass passing through unit area and thickness of membrane in unit time under the influence of unit hydrostatic pressure (Landis, 12). In the work presented here, we have measured the units of mass passing in a given time. While in the normal animal it may be assumed that the area and thickness of the filtering membrane did not change, this cannot be done with safety in the burned animal. At present, we can only guess at the area of the capillaries in the normal dog, and there are no estimates available of the area in shock. The only measurements of capillary blood pressure in shock that we

know of are those of Szanto (13). He found that the capillary blood pressure was decreased in surgical shock. It should be considered that the increase in rate of passage of protein may be due to increase in hydrostatic pressure or filtering area, as well as to increase in permeability per se. Without direct measurements of the first two factors, it is not possible to evaluate their significance at present.

It will be recalled that the animals were burned from the feet to the axillae. The increase in rate of passage of protein across the capillaries in the burned area is not surprising, although to our knowledge it has not been demonstrated before. But it is of even greater interest that the rate of passage of protein in the unburned area is altered in burn-shock. It should be emphasized that protein analyses of fluid from the burned area do not exclude extravascular sources of protein, nor have rates of passage usually been measured by this method. In our experiments, the horse protein could not have come from any site other than the vascular system.

The demonstration of a prompt alteration in permeability in the non-burned area is of great interest. Our results with total protein in lymph resemble those of McCarrell and Drinker (14) in histamine shock. They found "early in the period of shock a considerable amount of proteinized fluid escaped from the blood stream." On the other hand, in shock following hemorrhage, Fine and Seligman (15) state that they found no evidence of a significant loss of radio-active protein in the tissues after a moderately severe hemorrhage in dogs. The method reported here has not yet been applied in the conditions resulting from different types of hemorrhage, especially using a more extreme and prolonged hypotension than that employed by Fine and Seligman.

The fact that the change in permeability follows so shortly after the burn suggests the existence of a circulating toxic factor, although neurogenic and other possible factors have not been eliminated. In the past, the numerous failures to demonstrate a toxic substance have been largely efforts to demonstrate a vasodepressor action in normal animals, but the weight of recent evidence indicates that the lowering of blood pressure is a secondary change (e.g., Blalock, 16). A crucial experiment would be to test the effect of blood or lymph from the burned animal on the rate of passage of protein in normal dogs. The alteration of permeability in the non-burned area also raises the question as to how much this change may contribute to the development of shock. Data must be obtained on how permeability is altered at longer times following the burn before this can be evaluated.

The fact that we did not find a significant increase in the concentration of total protein in lymph following the burn has been mentioned. This is not contrary to the finding of increased concentration of horse protein, because the concentration depends on the movement of fluid as well as of protein. The evidence from rise in hemotocrit, rise in serum protein, rise in lymph flow, and initial decrease in concentration of lymph protein together point to increased filtration of fluid. Use of the total protein concentration of lymph as a measure

of endothelial permeability is, therefore, open to question. Landis (12) has emphasized that the original filtrate from the capillaries is modified by fluid movement. Furthermore, extravascular sources of protein in lymph in shock have not been excluded. In our experiments, the increase in rate of appearance of horse serum has certainly not been accompanied by an increase in total protein concentration in lymph.

The effect of anesthesia should be considered in connection with our results. It is well known that prolonged general anesthesia lowers the resistance of the animal to various forms of shock. The effect of the anesthesia per se on capillary permeability cannot be determined until the method is studied with animals under local anesthesia. In our experiments the anesthetic agents used were equivalent in the normal and burn-shocked animals.

Finally, the question may be asked whether data on horse serum in the dog are applicable to the normal protein of the dog. We have been unable to find data on the properties of dog serum protein. The physical properties of horse serum proteins resemble closely those of other mammalian species studied (Svedberg and Pedersen, 17). Antigenic differences are believed to be due to spatial arrangement of groupings, and not to a fundamental alteration of protein structure (see among others the recent work of Pauling and Campbell, 18). Finally, the marked increase in lymph flow accompanying only a slight fall in lymph protein concentration indicates that the total amount of native protein in the lymphatics of the dog is increased. We, therefore, feel that increased passage of horse protein across capillaries in the dog implies an increased passage of dog protein as well.

SUMMARY

1. A method is presented for using the rate of passage of horse serum protein across the capillary endothelium of the dog as a measure of capillary permeability.

2. The greater permeability of the capillaries draining the thoracic duct area than those of the cervical duct area is demonstrated quantitatively by this method in 3 normal dogs.

3. In dogs, following a burn up to the axillae by immersion in water at 72°C. for 60 seconds, there is a prompt increase in the rate of passage of protein, both in the burned area and in the non-burned area as well.

4. Values for total protein concentration in plasma, cervical and thoracic duct lymph are given for normal and burned animals. These did not demonstrate the permeability changes seen by the use of immunologically detectable horse protein.

5. The effect of the burn on hemoconcentration, rectal temperature and lymph flow is given. Intravascular hemolysis and escape of hemoglobin into the lymphatics is a consequence of severe burn.

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WATER SHIFTS IN DEEP HYPOTHERMIA^{1, 2}

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Within the past quarter century considerable information has been accumulated concerning the behavior of water in the body on exposure to cold environments. This has been derived chiefly from the results of reflex processes aroused by the action of cold on the body surface, the responses being in general of a protective nature.

Down to a very low degree of temperature the effects of environmental cold are such that the mammalian organism is able to maintain its own temperature levels within very nearly the normal⁴ limits. The mechanisms of heat production and conservation longest recognized all result in water shifting.

In response to cold the tendency is toward a centripetal movement of water from the surface of the body to the interior of the cells themselves. Increasing metabolism in such cells, as those of the muscle and liver, tends to attract water by osmotic pressure, and the familiar vascular changes associated with cold may play a further part in the shifting of water from the circulation to the tissue spaces.

That the above mentioned reflexes are mediated chiefly through the anterior hypothalamus has been shown in this laboratory (Barbour, 1940). It had, indeed, long been known that elimination of the function of the base of the brain, either by operative procedures or anesthesia, is inimical to temperature regulation. No attempt however has apparently been made to determine what water shifting may occur in the chilled body stripped of its normal defenses against cold, although it is common knowledge that the metabolic rate is, under such conditions, decreased chiefly in proportion to the fall in temperature.

Cold itself may result in eliminating the anterior hypothalamus as a protective influence. In this case as in all others where the body temperature has reached very subnormal levels, the condition is known as hypothermia.

Former studies of hypothermia. Medical history has been concerned with hypothermia in cases of exposure to snow and ice so intense as to overwhelm the regulation of body temperature. Such exposure has been frequently associated with acute alcoholism. Alcohol and other anesthetic substances have frequently had a like effect unassisted by unusual degrees of cold. The effects of such substances were studied by Simpson and Herring (1905) and Britton (1922) who reported on the elimination of reflexes (chiefly motor) and the order of the recovery of many of these. Hypothermia has been carefully studied in humans in attempts to use it therapeutically, notably by Smith and Fay (1941) in cancer

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² Aided by a grant from the Fluid Research Fund of the Yale University School of Medicine.

patients, and Talbott (1941) and collaborators in schizophrenia. Dill and Forbes (1941) have reported on metabolism studies on the last mentioned series of patients.

Hypothermia in therapy is accomplished with the assistance of anesthesia, for example by nembutal. It has however been considered of importance to study the effects of cold alone, applied with such intensity as to overwhelm all protective mechanisms. This is accomplished with relative ease in small animals, as in the classical studies of Walther (1862) who rendered rabbits powerless to resist cold of 18 to 20°C. by chilling their bodies to that temperature. J. B. Hamilton produced hypothermia in ice box environments in rats, mice and kittens with progressive descending paralysis of the central nervous system. When carried to the lethal point, death was attributed to respiratory failure from chilling the medulla. Neither he nor others have been able to produce a state of artificial hibernation, recovery from hypothermia being always possible in environments of 70°F or below. The experiments of Walther and those of Hamilton have the virtue of bringing out the action of cold as a depressant *per se*, and are free from complicating effects of drugs on reflexes which must be kept in mind in connection with the following mention of changes in water metabolism which have been reported in literature.

Previous findings on water shifting. Edema of the lungs in the rabbits was described by Walther as a terminal effect associated with congestion of the lungs, and watery serous exudates in the tissues and air passages. Rabbits dying after rewarming showed a serous pleurisy. Talbott and others (1941), in a body of a patient who had died of hypothermia, described a similar congested condition of the lungs. Woodruff (1941) described edema of the heart in dogs dying of hypothermia (in which nembutal anesthesia was used). One of his dogs exhibited pulmonary edema. He believes that the use of digitalis was beneficial in preventing mortality from hypothermia.

Blood concentration changes in hypothermia produced by the aid of anesthesia included increase in red blood cells, described occasionally by Smith and Fay in cancer patients. Similarly Woodruff describes increases in whole blood specific gravity in dogs near both the start and end of hypothermia. The initial increase in concentration was obviously the well known reflex response, while the terminal change was attributed to a shock-like condition. The reflex increase in blood concentration as measured by cell volume was seen in the first thirty-five hours.

Dilution of the whole blood is represented by acute anemia, described in some of the cases of Smith and Fay, but never until after one or two previous periods of refrigeration. In Talbott's case a slight blood dilution appeared near the end, that is, at the forty-fifth hour.

Changes in the serum appear to have been reported only by Talbott, Consolazio, and Pecora. The chlorides fell during the early hours of hypothermia, returning to the normal figure in the end. Serum protein gave a reverse picture being raised at first and lowered in the end, in these respects agreeing with the whole blood concentration. Tissue changes of interest were found by Talbott

and his collaborators. The percent of water was found below the average for normal autopsy subjects in muscle, liver, kidney, and brain, but increased in the heart muscle. The changes in sodium content suggested, however, a decrease in extracellular water inasmuch as sodium was below the normal average in all the organs just mentioned. Potassium was found increased in all these organs.

Object of this investigation. The above cited data are far from presenting a clear picture of the water metabolism in hypothermia, and our present intention has been to contribute toward filling this gap. Such investigations seem to be demanded by war conditions in the cold regions of the earth, and by a growing tendency to use cold as a therapeutic measure. The intention has been to determine the changes in water content of blood and various tissues, tracing, if possible, the movement of water in and out of the cells, and to gather information concerning the occurrence and mechanism of hypothermia. Possibilities of treatment of untoward conditions brought about by the above changes might thereby emerge.

PROCEDURES. Our studies have been conducted on monkeys (*Macacca mulatta*) and white rats, and all chilling procedures carried out in a room kept within a few degrees of 0°C. The body temperature of monkeys was lowered by direct removal of heat by a half inch copper coil through which water at about 7°C. was circulated by an electric motor. The padded cradle upon which the monkey was confined was strapped to the lower surface of this coil in such a way that the weight of the coil did not rest on the animal. As the coil extended only from the neck to the lower end of the trunk, it was possible to withdraw blood samples at any time from the femoral artery, and to follow the rectal temperature with an ordinary thermometer. Arterial samples were taken at the following body temperature levels: initial, 35, 30, and 23°C. When the last mentioned point was reached the monkey, still confined to the cradle, was removed to a room having the constant temperature of 28°C., and during the resulting rewarming the samples were removed at the same body temperatures in reverse order. The experiment was usually terminated when the animal reached a body temperature of 30°C. All blood samples were taken and centrifuged under oil; serum proteins were calculated from specific gravity determined by the Falling Drop Method, chlorides were determined by the method of Van Slyke and Sendroy (1923).

Rats were chilled similarly to monkeys by a one-eighth inch coil over which they were strapped in a prone position. Studies were also made on rats simply exposed to a cold room at 3-4°C., confined only by individual cages, in order to study the reflex effects of cold. Such exposures lasted one hour. Following the above procedures heart's blood was removed while the rat was still in the cold room, and serum proteins and chlorides determined as above.

For tissue studies many of these rats were killed by rapid strangulation after either of the above procedures, that is, in a condition of hypothermia, or with protective reflexes against cold. The rats were skinned and both water and chloride determinations made upon brain, muscle, liver, skin, lungs, and samples of the remainder of the animal after mincing and thorough mixing. Water was determined by drying in an oven at 105°C., and chlorides by the wet ash method of Sunderman and Williams (1921). Metabolic changes in response to cold reflexes and hypothermia were determined in the cold room in some rats by the Haldane Train Method in which the carbon dioxide was fixed by shell caustic, and the water by concentrated sulfuric acid.

Many rats were chilled to body temperatures of 16°C. and then removed to the constant temperature warm room at 28°C. where the recovery of temperature and reflexes and gross edema changes were followed.

RESULTS. *Monkeys.* Chilling by the coil method produced a practically constant fall of the rectal temperature of the monkeys at the rate of about 5.5°C . per hour (fig. 2). During the first hour or two the usual reactions to a cold environment were in evidence with shivering of the skeletal musculature, and paling of the body surfaces. When the body temperature had fallen below 30°C . the general muscular relaxation of the animal betrayed the depressing effects of cold upon the central nervous system. When a monkey at 23°C . body temperature was removed to the 28° room its temperature gradually returned toward normal, but at a somewhat slower rate than it had fallen, namely, 2.74°C . per hour (fig. 3). Many reflexes were found slow, including the pupillary reflex to light. Pain responses to pin pricks were in general abolished. The grasping reflex was not found below 30°C . The pupillary reflex to light gradually regained its normal

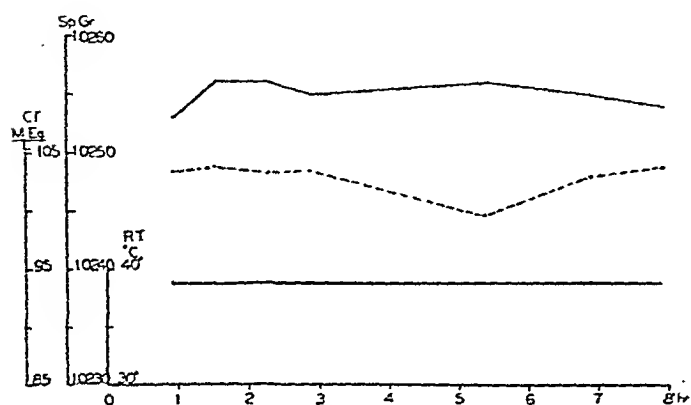


Fig. 1

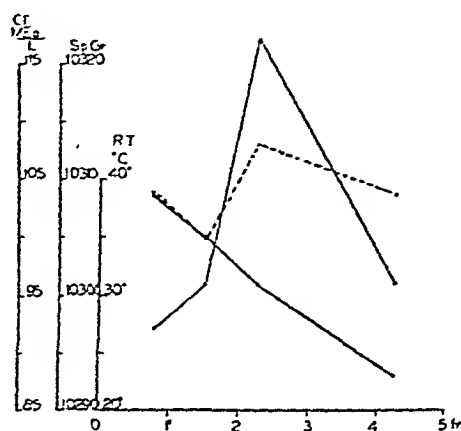


Fig. 2

Fig. 1. Serum specific gravity and chloride changes and temperature curve of untreated monkey. — Rectal temperature, ---- Chlorides, — Specific gravity. Monkey 16.

Fig. 2. Serum and temperature changes in monkey during chilling. — Rectal temperature, ---- Chlorides, — Specific gravity. Monkey 11.

speed. If any edema is present in monkeys at these low body temperatures, we were unable to establish it definitely, although some apparent swelling was noted about the shoulders and forearms in one case.

Serum proteins and chlorides. A well trained monkey confined to a padded cradle for the length of time usually occupied by our chilling and rewarming experiments, can tolerate the extraction of many blood samples from the femoral artery without significant change in proteins and chlorides. A control experiment of this sort is illustrated in figure 1, showing the taking of blood samples at intervals corresponding to those in our chilling and rewarming experiments.³

While a monkey was being chilled to a body temperature of 23°C . the serum protein and chlorides followed a fairly constant pattern which is typified by figure 2. There was constantly an increase in serum protein, reaching a peak usually at or before the temperature had fallen to 30°C . This represents the well

³ Average from blood samples taken at like intervals from 12 monkeys were in percentage changes from original sample: for chlorides, 1.5, 1.4, 0.03, 1.8, -1.7, 2.1; for specific gravity, -0.7, -1.3, -1.7, -3.3, -3.5, -1.8.

known anhydremic reflex response to cold. Serum chlorides also increased during this initial period, in conformity with the well established osmotic increase (Barbour and Gilman, 1934). The chloride peak, however, was usually preceded by a depression. This preliminary fall in chlorides, while unexplained at present, resembles very much certain unpublished findings in dogs. These animals when given continuous injections of minute quantities of epinephrine, showed a rise in serum proteins, as in the cooling process, which is accompanied at first by a fall in serum chlorides. It is therefore possible that the preliminary depression on cooled monkeys is an epinephrine effect. The blood sample taken at a body temperature of 23°C. constantly showed a reversal in the serum changes.

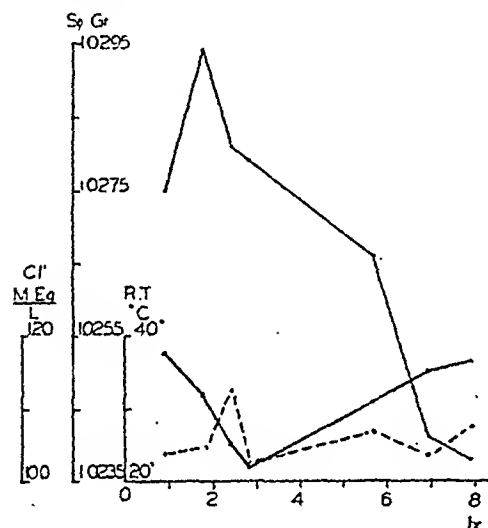


Fig. 3

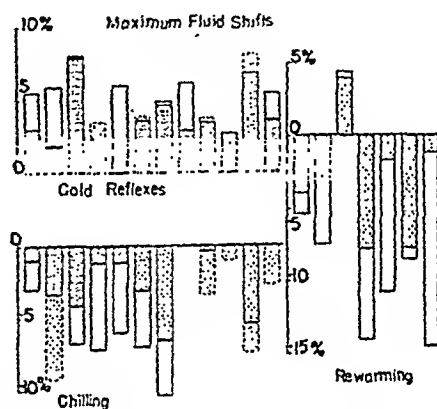


Fig. 4

Fig. 3. Serum and temperature changes in monkey during chilling and rewarming. — Rectal temperature, ---- Chlorides, — Specific gravity. Monkey 4.

Fig. 4. Serum changes in monkeys interpreted as fluid shifts. Percentages are in terms of the level at the beginning of an experiment. Unbroken end of columns indicates total fluid change (measured by sp. gr.), dotted portion of columns, changes in cell water (measured by Cl'). First group shows maximum reflex effect of cold. Second group (chilling) shows amount of reversal shift from previous maximum. Third group (rewarming) shows amount of continued loss calculated from 23°C. level to point of maximal serum hydration.

Protein and chlorides approached, or attained, the original levels. On rewarming from 23°C. body temperature the serum proteins did not reverse again but continued the downward course, often with such a tremendous decrease as shown in figure 3. This dilution tendency is, of course, typical of the response to warming animals up from normal temperature before any dehydrating factor such as sweating appears. The chlorides did not show the extensive decreases during the rewarming process, but tended to remain nearer the initial levels than did the proteins. The final chloride shown at about 35°C. is typical of the usual course.

Interpretation of serum changes in terms of fluid shifts. The extent of the serum changes in each of our first twelve monkey experiments is illustrated in figure 4, the changes being referred to as fluid shifts. The percentile changes in proteins

are represented by the maximal length of most of the columns, and the chlorides by the length of the dotted portion of the columns. The group entitled cold reflexes was compiled from the peak changes in each experiment during the chilling process, these being taken to represent the maximal protective effects against cold. The group marked chilled was compiled from the individual differences between peak effects and status at 23°. The group of changes entitled rewarming represents the maximum percentile decrease in protein or chloride from the level at 23°C.

The movements of fluid in the body throughout the whole procedure of chilling and rewarming are possibly best interpreted from the average fluid shifts (fig. 5).

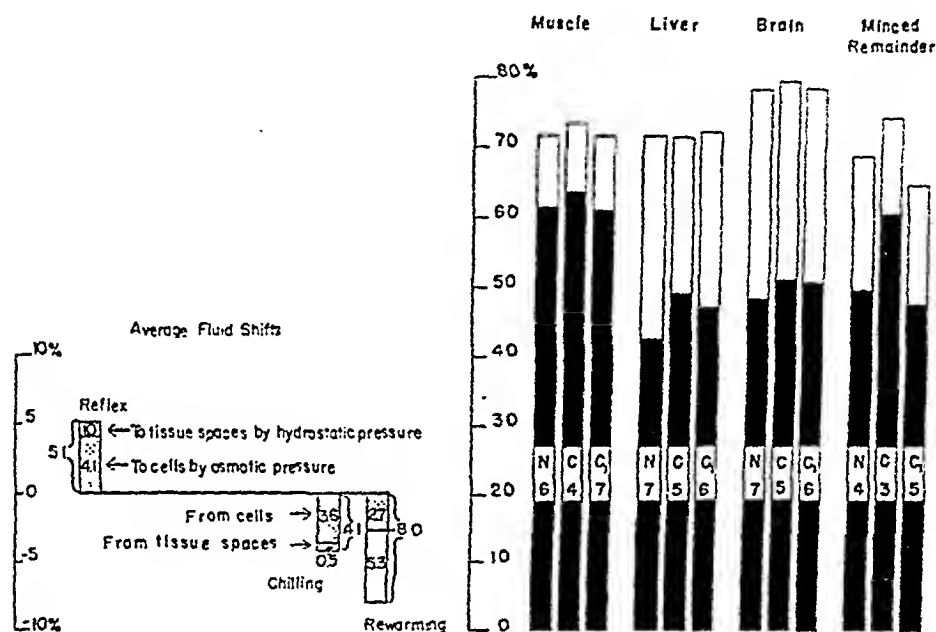


Fig. 5

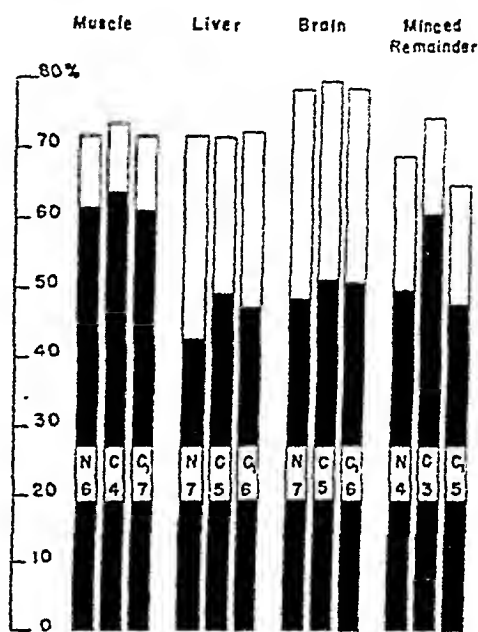


Fig. 6

Fig. 5. Average in fluid shifts in monkeys shown in figure 4.

Fig. 6. Total and intracellular water of tissues of chilled rats. Height of columns, total water, black portion, intracellular water. N = normal, C = cold, with reflexes intact, C₁ = deep hypothermia with loss of reflexes. Figures show number of cases averaged for each column.

The serum protein is of course an indication of the total fluid content of the serum, whereas the chlorides may be regarded as indicating the movement of water in and out of cells, thus showing the well known protective responses to cold. The chloride portion of the first column in figure 5 represents a 4.1 per cent movement of water into body cells. This on the basis of osmotic pressure studies made by Barbour and Gilman (1934), we have always assumed to be due to increase in osmotic pressure resulting from the increased metabolic rate of the cells as in shivering, etc., which must produce a temporary accumulation of metabolites. The total height of the columns represents 5.1 per cent loss of fluid from the blood (as judged by protein and specific gravity) leaving a 1.0 per cent loss to be accounted for otherwise than by metabolism. The extra loss of water we

interpret as the net pressure effect associated with vascular responses to cold; fluid is of course lost to the tissue spaces as the net result of increases in hydrostatic pressure in the capillaries.

The entire water shifting picture becomes reversed by chilling to 23°C. which evidently produces such a depression of the central nervous system that the water shifting reflexes, above referred to, are no longer possible. This reflex reversal therefore, is ascribed to functional exclusion of the anterior hypothalamus by cold. The second column in figure 5 shows that the total water gained by the blood during this reversal is 4.1 per cent of the original serum water of which 3.6 per cent may be considered as coming back from the cells, the remaining 0.5 per cent being gained from the tissue spaces by a reversal of the hydrostatic pressure conditions. In other words, depression of the anterior hypothalamus by cold has reversed the metabolic and vascular changes which accounted for the fluid shifts. The third column represents the average results of rewarming from 23°C. The total gain of fluid by the blood averages 8 per cent. The greater part, 5.3 per cent, apparently comes directly from the tissue spaces because only 2.7 per cent, as judging by the chlorides, appears to be released by the cells; or the hydration may be chiefly relative due to the escape of protein.

It is difficult to explain the original source of such a large fluid gain by the blood. We must assume in the absence of nervous function that increasing temperature acts directly upon the arterioles and capillaries in such a way as to lessen intracapillary pressure throughout the greater part of the organism. One may well believe that the same processes are responsible for the dilution of the blood which occurs upon ordinary exposure to mild degrees of heat before such dehydrating processes as sweating set in. As a matter of fact, under the latter conditions it has been found that decrease in specific gravity of the blood is usually greater than decrease in osmotic pressure (Barbour, 1940); thus we see in the rewarming period of the experiment a picture of the direct action of increasing warmth in the absence (until higher temperatures are reached) of any reflex mechanism.

Rats. General effects of cold. In the early stages of exposure to cold our rats, like other mammals, exhibited shivering although the time of its occurrence as well as its anatomic distribution was subjected to considerable variation. As the body temperature descended to 20°C. and below, the general depression of the animal as in monkeys, was evidenced by complete muscular relaxation. At 16°C. the rats were completely prostrated in a condition resembling deep anesthesia. At this point considerable edema of the face, eyelids, and jaw as described by Hamilton, was a constant finding.

Rewarming in the 28°C. room usually served to exaggerate this edema so that by the time the animal attained a temperature of 30°C., the head often appeared cone shaped. The recovery of reflexes was in a sequence similar to that described by others.

Total metabolism. The effects of cold on the metabolic rate of rats were found very much as expected. All of the experiments were made in the cold room at 3 to 4°C. and when the rats had been cooled only to a body temperature of 26°

to 30°C., a marked increase in metabolic rate was observed, whereas when chilled to the state of complete depression with body temperature 18–20°C., the metabolism was found greatly reduced as in the patients described by Dill and Forbes. Many of the effects were largely influenced by the amount of visible muscular tension or movement. Seven rats at the upper temperature range mentioned produced on an average 7.78 calories/kilo/hour as contrasted with an average from 26 normal rats (done at room temperature 28°C.) of 5.74 calories/kilo/hour. Six rats chilled below the point of cold reflexes showed a heat production of only 3.65 calories/kilo/hour. Thus in the initial period of cooling our rats exhibited an increase of 37 per cent, but in hypothermia a loss of 36 per cent.

Blood changes. On chilled and rewarmed rats the changes in blood proteins and chlorides evidently follow the same pattern as those in monkeys; in support of this, the following findings may be cited.

Proteins were calculated from the specific gravity findings, the average normal for 4 rats being 7.4 per cent. Four rats of the same age as the normals, when exposed to the cold room at 3 to 4°C. for one hour, showed an average protein content of 7.6 per cent. The average effect on 4 rats chilled on a coil to a body temperature of 16° was to reduce the protein to 6.8 per cent, while in 11 rats rewarmed from 16°C. to 30°C. the marked hydremic effect seen in monkeys was reflected in an average protein content 6.7 per cent. The percentage change from normal of dehydration and hydration effects was as follows, blood dehydration on initial exposure to cold 1.4 per cent; hydration in profound hydremia, 5.9 per cent; hydration of serum above normal on rewarming, 7.3 per cent.

The average serum chloride for the 4 normal rats mentioned above was 102.8 m.eq./liter, whereas in the 4 rats exposed to the cold room, the average chlorides rose to 104.7 m.eq./liter. Eight rats chilled to an average 18.2° showed a chloride figure 102.1 m.eq./liter illustrating a reversal of the reflex rise similar to that found in chilled monkeys. On rewarming rats from 16° to 30°C. no further change in chlorides was consistently demonstrated. An average of the above mentioned 11 rats yielded 101.0 m.eq./liter which may indicate a slight decrease as found in monkeys.

Changes in water and chlorides in rat tissues. The tissues from which our data are here reported were all taken from rats varying from 67 to 161 days of age. The chlorides, percentage total water, and its intracellular portion in terms of fresh tissue are summarized in table 1. In this table the number of cases concerned in each procedure with a given tissue follows the data for each tissue; exceptions to this are given in parentheses.

The intracellular water is calculated from the chloride content of each tissue and of the serum of the animal concerned. In the muscle the average will be seen to have increased from 60.66 per cent to 63.74 per cent on exposure to cold without loss of reflexes. On chilling with the coil to the completely depressed state the intracellular water returned to 61.08 per cent. For the liver the respective figures are 42.78, 49.08 and 47.24 per cent. The same tendencies were shown in the brain but to a lesser degree, the percentages being 48.51, 51.17 and 50.82. The same general trend is strikingly brought out in the minced remainder

of the rat in which the initial period of cooling raised the intracellular water percentage, 49.77 to 60.73, while the extreme chilling reduced it to 47.66 per cent. The changes in the above tissues are illustrated in figure 6.

The total water of the various tissues follows the same trends as the intracellular water except in the case of the liver for which no explanation can be offered at present. The total water of the lung in deep hypothermia was found to be 81.88

TABLE 1
Tissue chlorides and water percentages

	CONTROL	ACUTE COLD OVER ICE IN COLD ROOM —REFLEXES	CHILLED ON COIL IN COLD ROOM		CONTROL	ACUTE COLD OVER ICE IN COLD ROOM —REFLEXES	CHILLED ON COIL IN COLD ROOM
Average rat temperature				Average rat temperature			
	37.5°C.	22.4°C.	18.9°C.		37.5°C.	22.4°C.	18.9°C.
Muscle				Total water			
Chloride	11.82	9.70	8.76	(per cent)....	78.22	79.28	78.49
Intracellular				No. of cases....	7	5	(6)
water (per				Minced remainder			
cent).....	60.66	63.74	61.08	Chloride.....	21.36	13.73	18.31
Total water				Intracellular			
(per cent)....	71.90	73.08	71.80	water (per			
No. of cases....	6	4	7	cent).....	49.77	60.73	47.66
Liver				Total water			(4)
Chloride	30.65	23.31	22.18	(per cent)....	68.57	74.22	64.41
Intracellular				No. of cases....	4	3	5
water (per				Lung			
cent).....	42.78	49.08	47.24	Chloride.....	52.31		52.50
Total water			(4)	Total water			
(per cent)....	71.90	71.60	72.01	(per cent)....	77.89		81.88
No. of cases....	7	5	6	No. of cases....	5		3
Brain							
Chloride	31.31	29.01	30.38				
Intracellular			(5)				
water (per							
cent).....	48.51	51.17	50.82				
			(3)				

per cent as contrasted with a normal of 77.89 per cent. This organ was found very congested and edematous as described by others for deep hypothermia.

The above evidence from determinations of chlorides and water in rat tissue confirms the deduction made from the studies of chloride concentration of the blood. That is to say that, as a part of the reflex response to cold in the initial period of chilling, the cells take up very appreciable amounts of water. When the nervous system, however, becomes depressed by the cold, this process is seen

to be reversed. The intracellular water changes in muscle, liver, and minced remainder afford particularly good evidence for this.

DISCUSSION. The primary and secondary effects of gradual chilling upon water movements in the body now appear clearly established, and it is interesting to see how well the fragmentary observations in the literature fit into the scheme we have outlined. There can be no set rule as to just when the reflex anhydremia will disappear, but its occurrence is evidenced in the red cell increase reported in the patients of Smith and Fay, and the serum protein increase in Talbott's patient where the concentration rose from 6.5 to 7.2 per cent in the first 7 hours, and was maintained at about this level until the 35th hour. It is seen also in the whole blood increase in Woodruff's dogs.

The secondary shift, after abolition of the defenses against cold, is illustrated by the acute anemia of Smith and Fay's patients late in the course of refrigeration where appreciable decreases in hemoglobin and red blood cell counts were reported. Such an anemia was not reported in Woodruff's dogs although it might have been expected from the nembutal which was given. In Talbott's patient it seems to have been shown in the fall from 7.1 per cent serum protein in the 35th hour to 6.2 per cent in the 45th.

An analogy to our results appears in the various tissue determinations in Talbott's patient as described above where the total water of many of the individual tissues was found lower than in the normal subjects. These tissues in hypothermia showed less than the normal sodium content; this may indicate that the water taken by the cells in reflex response to the cold had probably not yet been released.

The high degree of hydremia noted on rewarming both monkeys and rats after hypothermia has not been definitely explained but undoubtedly bears a relation to the edema constantly found in rats. As it is difficult to account for so large an accumulation of water in the serum in proportion to the original amount, it might be concluded that the serum probably becomes relatively diluted through loss of protein which in its turn would support the edema. This point remains to be determined. The matter is of considerable importance in view of the possible employment of hypothermia in the treatment of shock which might be of definite value if the dilution represents an absolute hydration of the blood; contrariwise, if it represents an escape of protein.

The possible control of edema by the use of certain drugs will be discussed in another paper.

SUMMARY. Monkeys chilled to 23°C. at a rate of body temperature fall of 5.5°C. per hour showed the following changes: 1, in the initial period with retention of protective reflexes, average maximum increase in serum protein of 5.1 per cent and chloride 4.1 per cent; 2, at end of chilling period (with neuro-muscular depression) an average reversal from the peak was found in serum proteins mounting to 4.1 per cent, and in chlorides of 3.6 per cent, of the normal level; 3, on rewarming at a rate of 2.74°C. per hour, these reversals in the serum fluid were continued amounting to a maximal decrease in serum protein of 8 per cent and chloride of 2.7 per cent of the respective original levels.

Rats exposed to a room temperature of 3 or 4°C. for about one hour, whether kept in individual cages or confined in proximity to ice, exhibited: 1, the usual reflex responses to cold, especially shivering and muscular rigidity; 2, an increase in metabolic rate of approximately 40 per cent; 3, increased serum proteins and chlorides; 4, in certain tissues, liver, muscle, brain and minced remainder of skinned rat, increases in intracellular water.

Rats chilled to 16°C. within about one hour showed the following changes: 1, complete neuro-muscular depression with partial edema of the face, jaw, and eyelids; 2, a metabolic rate approximately 40 per cent below normal; 3, serum protein and chloride levels showing reversal from the concentration consistently seen in the initial stage, as in monkeys; 4, tissue changes in intracellular water also indicating reversal of the initial reflex effect which was shown by muscle, liver, brain, and minced remainder of skinned rat.

CONCLUSIONS

Exposure to cold with retention of protective reflexes leads to a gain of intracellular water throughout the body. When, however, the central nervous system becomes so chilled as to cause general neuro-muscular depression, the effect on the hypothalamus is to abolish the reflex responses to cold, which process includes a reversal of the water shift with increased extracellular fluid. Subcutaneous edema tends to occur and is augmented on rewarming the animal, which procedure increases greatly the hydration of blood, at least relatively.

We are indebted to Mr. Lawrence Brigham for the determination of total metabolism in rats.

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FURTHER STUDIES ON THE WATER BALANCE OF FROGS

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In a paper published in 1921, the author (1) demonstrated that frogs, when kept in water and injected with extracts of the posterior lobe of the pituitary body, show a temporary increase in body weight. This gain in weight varied between 6 to 28 per cent of their original body weight and lasted for some hours. It was found that during this period frogs did not excrete less urine than they had before the injection of the extract. It was further found that nephrectomized frogs when injected with pituitary extracts gain 6-8 grams in a few hours. Nephrectomized frogs of the same size, but not injected, gain 2 to 3 grams daily in the first days after the operation. Therefore, it was concluded that the gain in weight of frogs following the administration of post-pituitary extracts was due to extrarenal factors.

These results were confirmed by Biasotti (2), Jungmann and Bernhardt (3), Heller (4), Steggerda (5), Novelli (6), Steggerda and Friedman (7), Steggerda and Essex (8), Rey (9), Boyd and Brown (10), Boyd and Gibson (11), etc., and numerous important contributions were made by these authors which threw light on the reaction involved. The response elicited by the previous communication aroused the author's further interest in the problem of the water balance of frogs. As a result it was decided to study the influence of drugs which are known to have an antidiuretic, pituitrin-like effect on mammals or man. Such drugs are anesthetics and narcotics as demonstrated for mammals by Molitor and Pick (12), Bonsmann (13), and for man by the author (14).

The author's (unpublished) observation that frogs, injected with commercial extracts of the posterior lobe of the pituitary body become very drowsy, and, with the use of larger doses of the substance become almost narcotized, served as a further stimulus. Only one reference to a similar observation was found in the literature. Boyd and White (15) state that frogs after repeated injections of Pituitrin (P. D. & Co.) became "less active" and explained the condition as "water intoxication". In the author's experiments the drowsiness of the frogs preceded any appreciable gain in weight. Two possibilities had to be considered: the phenomenon could result from an anesthetizing effect of the extract proper or be due to its content of chloretone which is added to most extracts as a preservative.

METHOD. The same technique was used as in 1921. Frogs, *Rana pipiens*, weighing 30 to 50 grams were placed individually in 2 quart glass containers with 200 cc. of tap water. Each container was covered with a metal lid perforated with about a dozen holes. The weight of each frog was noted after careful drying and catheterization in one hour or one-half hour intervals. The anesthetics

used were: alcohol, ether, chloroform and chloretone. Ten cubic centimeters of a 95 per cent alcohol solution, or 2 cc. of ether, or 0.5 cc. of chloroform were added to the 200 cc. of tap water in which the frogs were immersed. The water was usually changed after one hour. A 0.5 per cent solution of chlorbutanol was used. One-tenth to 0.2 cc. per 10 grams of frog was injected into the lymph spaces. The frogs were weighed to the nearest 0.1 gram, the injections made with a tuberculine syringe. The catheter used was constructed according to Morishima (16).

Protocols. Morphine in doses up to one centigram per 10 grams of frog appeared to have no effect on the water balance of the animals. The experiments were discontinued.

A. The effect of the aforementioned anesthetics on the body weight of frogs is a uniform one. During the hour in which the frogs were immersed in the anesthetic there was a slight gain in weight; in the following hour when the frogs were again placed in tap water the gain in weight was even greater. The peak of the body weight usually was reached in 2 to 3 hours. The magnitude of the gain in weight depends not only upon the concentration of the anesthetic, but also upon the length of time the animals were kept in the solution. The average gain in weight of frogs immersed in ether or chloroform was about 4 per cent of their original weight varying between 3 to 5 per cent. The average gain in weight of frogs kept in a 5 per cent alcohol solution was 6 per cent, varying between 2 to 15 per cent. The frogs injected with 0.1 cc. of a 0.5 per cent solution of chloretone per 10 grams frog gained an average of 1.5 per cent varying between 1 to 2 per cent. These figures are derived from 9 experiments each with ether, chloroform and chloretone and from 57 experiments with alcohol.

B. Twelve experiments were made on pithed frogs. When the operation wounds were closed up and the animals weighed, 6 of the pithed frogs immediately were immersed in a 5 per cent alcohol solution for one hour, while the other 6 pithed frogs were immersed in tap water thus serving as controls. The pithed frogs in alcohol gained 6.4 per cent of their original body weight in 2 hours while the controls gained an average of 4.6 per cent in the same period.

C. Nine frogs were nephrectomized. The day following the operation the animals were immersed in a 5 per cent alcohol solution for one hour. The average gain in weight of the 9 frogs was 1.8 grams in 2 hours as a result of anesthesia.

D. The following series of experiments was undertaken to obtain information as to the elimination of the water accumulated in the body of the frog following anesthesia. In animals surviving the anesthesia one notes that the body weight returns to normal in a few hours. Therefore, one can distinguish a first phase in the reaction to anesthesia in which the animals gain weight, and a second phase in which the body weight returns to the original level. In order to ascertain the beginning of the second phase, that is, the time the peak of body weight is reached, more frequent weighings were made until the point at which the frogs began to lose weight became evident. Then the experiment was continued in the following way. When, e.g., 6 frogs had been simultaneously immersed in alcohol and the second phase was reached the cloaca was closed in 3 of these frogs with

tobacco pouch sutures. It could be observed that these operated frogs again began to gain weight while the controls lost weight eliminating large quantities of urine similar to water. When the sutures of the operated frogs were opened and the frogs catheterized the amount of urine found showed that all the accumulated water in the body had been eliminated through the cloaca.

E. To see the response of the kidneys in normal and anesthetized frogs tap water in the amount of 10 per cent of the body weight was injected into their lymph spaces. Normal frogs began to lose weight immediately after the injection. They again reached their original body weight in about 2 hours. Under the same conditions frogs subjected to alcohol anesthesia and injected with additional water show a slight gain at first. Loss of water begins after 1 to 2 hours; the original body weight is reached in about 3 hours.

F. It then appeared obvious that a study of the effect of a combination of pituitary extract and alcohol anesthesia on the water balance of frogs was indicated. Frogs were immersed in a 5 per cent alcohol solution for one hour. Upon their recovery from the anesthesia one unit of Pitocin (P. D. & Co.) per 10 grams of body weight was injected. Anesthetized frogs to which Pitocin was administered gain much more weight than normal frogs did after the same dose of Pitocin. The combination of alcohol and Pitocin produces gains in body weight that are equivalent to the sum of the gains produced by each drug administered individually.

In these experiments pituitary extracts were injected simultaneously with the recovery of the frogs from their anesthesia in order to meet the objection that pituitary extracts simply retain alcohol like water in the body thus explaining the greater effect of the 2 drugs administered simultaneously. It was noted that frogs just recovering from alcohol anesthesia, when injected with Pitressin (P. D. & Co.), or Pitocin (P. D. & Co.) became anesthetized again, and remained narcotized for at least one hour longer.

In most of the commercial extracts chloretone is added as a preservative in a 0.5 per cent solution. There is, therefore, 0.005 gram in 1 cc. of the solution. The dose of chloretone which produces anesthesia in mammals is about 0.15 gram per kgm. body weight. If one considers that 0.005 gram of chloretone is injected with 1 cc. of Pituitrin into a frog weighing 30 grams one must realize that that represents 0.15 gram chloretone per kgm. body weight. When using one or two units of these extracts per 10 grams of frog one approaches the dosage of chloretone that is expected to exert an anesthetic effect on frogs.

As a matter of fact using a 0.5 per cent solution of chloretone and injecting 0.1 to 0.3 cc. per 10 grams of frog one notes that the animals behave exactly in the same way as after a corresponding dose of pituitary extract that contains chloretone as a preservative. If alcohol anesthesia is combined with the administration of chloretone the anesthesia is definitely prolonged and results in a greater gain in weight.

According to information forwarded by Burroughs, Wellcome & Co. their "Infundin" does not contain chloretone or any other preservative. Frogs injected with Infundin remain as lively as they were before the injection.

DISCUSSION. 1. Is the influence which alcohol, ether, chloroform and chloretone exert upon the water balance of frogs a true biologic reaction, or are the

phenomena due to dissolution of the lipoids in the skin of the frog? The answer is there could not be a rapid reversibility of the reaction if the action of the anesthetics depended on dissolution of lipoids.

2. Can we explain the gain in weight of anesthetized frogs as an effect upon their central nervous system? Pithed frogs when immersed in alcohol gain slightly more weight than the controls do. This finding makes one believe that the central nervous system can not be the only factor involved. Furthermore it was observed that frogs continue gaining weight for one hour longer after they have awakened from the anesthesia (17). It is conceivable that the muscles of the lymph vessels present in the skin of frogs (18) are influenced by anesthetics. A dilatation of these lymph vessels would permit a greater amount of water to enter the body.

3. Is the gain in weight due to an increased intake of water, or to a diminished output, or to a combination of both? An increased intake as a result of the anesthetic has been established by experiments on nephrectomized frogs: If nephrectomized frogs are immersed in alcohol one notes a superimposed steep ascent of the weight curve.

Frogs of 30 to 50 grams are believed to excrete 3 to 4 cc. of urine in 24 hours. A further reduction of the urine formation can not play an important part in the gain in weight in the first phase of the reaction. Regarding the abundant urine formation in the second phase of the reaction one is under the impression that the response of the kidneys to increased intake might be termed delayed. There are no means of knowing how quickly a response from the kidneys could be expected after an increased intake of fluid via the skin. It is noted, however, that additional water injected into the lymph spaces leaves the body much more promptly in normal frogs than in frogs that have been subjected to anesthesia.

It may be worthwhile at this point to review the main difference of the action of post-pituitary extracts on the water balance of mammals and amphibians. In mammals the antidiuretic effect of pituitary extracts becomes manifest only after the administration of additional water. Without the forced administration of water in mammals or without voluntary previous intake of additional water in man, the pituitary extracts have hardly any antidiuretic effect (19). Under normal conditions frogs do not drink water. Normally water passes through their skin into the body, a process which is apparently entirely involuntary. The increased intake of water is an experimental premise if we want to demonstrate the influence of pituitary extracts in man or mammals but it is part of the reaction to these extracts in amphibians. At any rate, enormous doses in comparison with the body weight of frogs are required to produce the gain in weight, a fact which deserves further investigation.

SUMMARY

1. Anesthesia in frogs influences the body weight in a similar way as do extracts of the posterior lobe of the pituitary body.

2. Alcohol anesthesia in combination with pituitary extracts results in an increase in the frogs' body weight which is equal to the sum of the constituents.

3. In alcohol anesthesia the first phase of the reaction is due to increased intake

of water and probably a delayed response of the kidneys. The second phase is a 100 per cent renal accomplishment.

4. The content of chloretone present in some of the commercial extracts is high enough to influence the weight curve and is even responsible for the anesthetizing effect of the extract.

I wish to express my gratitude to Dr. Albert A. Epstein for rendering these studies possible, his guidance and advice, and to Dr. V. de Beck for valuable assistance in writing this paper.

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DO RATS THRIVE WHEN DRINKING SEA WATER?

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While many species of mammals might regularly drink sea water, there appears to be no proof that they do. It seems important to know whether any voluntarily ingest it when no fresh water is available; if so whether they are able to excrete the salts taken in it; and whether they survive, while drinking it, longer than when without any source of water. Questions of particular import are: does the urine acquire a concentration sufficiently greater than the intake so that some net water of intake remains available to the body? and, is maintenance of water content by drinking sea water possible after preliminary reduction of that content?

Procedures. Albino rats were chosen for the experiments because they are known to form highly concentrated urine. Males were kept in individual wire cages which allowed urine to collect into measuring cylinders. Dry food, furnished *ad libitum* in a deep beaker, was, in some tests, whole dried milk slightly mineralized with iron, copper, and manganese (Kemmerer *et al.*, 1932). This food is about 27 per cent protein, and hence required considerable water for its metabolism. In other tests dog chow was supplied. Drinking water was furnished in an inverted burette, or cylinder, so arranged that none of the water could drain into the urinary collector without passing through the rat. The chief quantities measured were: body weight, water intake, food intake, urinary output, urinary specific gravity, urinary chloride concentration, and survival time.

WATER AND SALT METABOLISM. Turnovers. Rats allowed dried milk and tap water *ad libitum* consumed 4.01 per cent of the body weight (B_0) of food daily (77 days on 7 individuals). This food had only 2 per cent of water in it (0.1 per cent of B_0 per day), but by oxidation formed 2.5 per cent of B_0 per day. Of water, 14.6 per cent of B_0 was drunk per day. Altogether 0.84 gram of water was available per potential Calorie of food consumed. One milliequivalent of chloride was ingested and excreted daily per 100 grams of rat. About 0.5 per cent of net weight was gained each day by the 200 gram male rat. Upon the milk diet the loss of water in urine was unusually great (10 per cent of B_0 per day) and the evaporative losses amounted to about 8 per cent of B_0 per day. These data are the basis of later comparisons.

Ingestion of sea water. When given sea water in place of tap water, body weight was lost. An important feature is that certain individuals lost weight more slowly than others. Some of those individuals also ate more and drank more than the others. The ones that drank least lost weight at the same rates as rats that had no water available; none lost significantly faster. In other words,

rats that kept the body weight high did so by ingesting more, not by losing less from the body.

The chief factors in loss of body weight are, therefore, the failure to take food in usual amounts and the failure to take water in sufficient amounts to replace the materials lost. The question at issue is, would the animals maintain themselves if their urges (appetites) were such as to lead them to take more of each? The answer is no; it seems to be given in part by the character of the urines excreted. Those that drank more sea water, formed urines of somewhat higher concentration of total solids and of chloride. The differences among individuals

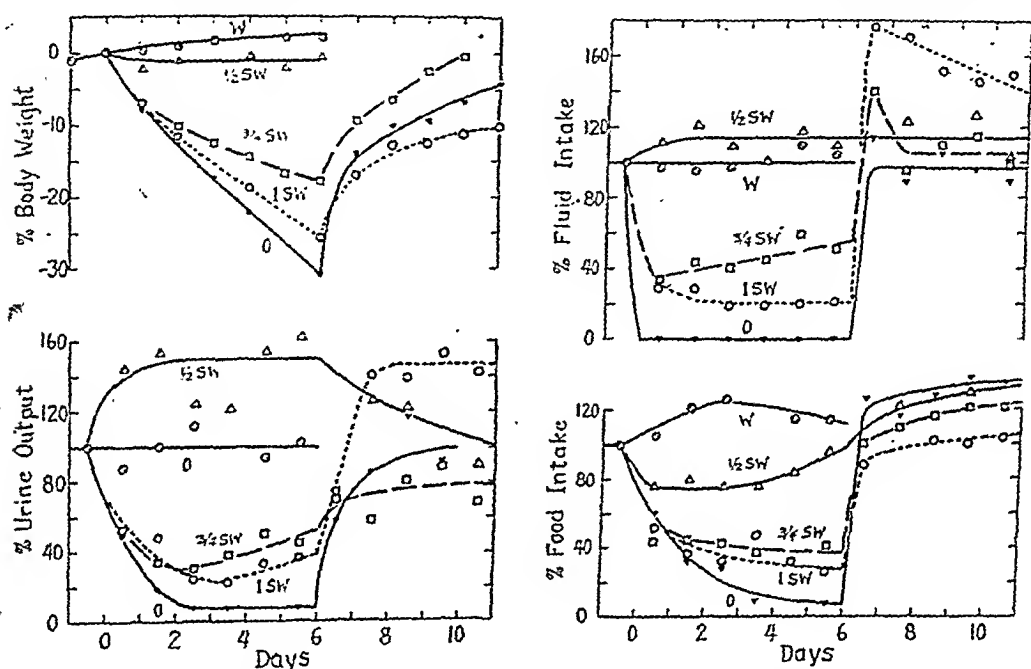


Fig. 1. Increments of body weight (in per cent of initial weight) and relative exchanges (each in per cent of its control rate) in rats maintained for 6 days with various concentrations of sea water to drink, SW, or with fresh water to drink, W, or no drink, O, and then allowed to recover with fresh water to drink in the after-period. Three to seven tests are averaged for each point. Dry milk was always available *ad libitum*. Body weight, urinary output, fluid intake, and food intake are separately indicated.

were in part, it seems, such as allow sea water to be metabolized by some rats and not by others. Four individuals at times formed urines containing 0.60 M chloride, while three others never attained concentrations above 0.52 M in the same sorts of "concentration tests." The sea water, which was collected at Woods Hole, Mass., contained 0.52 M chloride. Under some conditions, therefore, certain rats were able to utilize for purposes other than excretion about one-fifth of all the sea water drunk.

This one-fifth was not, however, enough to maintain body weight, the data show. Part of the deficit of body water represented the fact that much less fluid was regularly drunk than when tap water was available (fig. 1). This may imply that water can be utilized only slowly under these difficult circumstances. It

also draws attention to the fact that in the rat, most water is being lost not in urine but in evaporation. The latter rate of loss appears to be little diminished during dehydration; it continues regardless of the body's water content. Evidently, the rat's unique ability to spare some water by producing highly concentrated urine is overpowered by its natural inability to spare water from evaporation. When the rate of evaporation is added to the rate of excretion, the rat (with its rapid evaporation per unit weight) is little if any better off than man, who produces urine less concentrated than the rat does (maximal 0.37 M Cl for men). The fact remains, however, that upon days when the rats drank most sea water they lost least weight.

When sea water was furnished which was diluted to $\frac{3}{4}$ strength, the same variation of results was obtained; some individuals lost weight more slowly than others, but again none maintained their original weights. After some days, however, a depleted body weight could in one of five individuals be kept stationary. Upon $\frac{1}{2}$ strength, the original weight was maintained (fig. 1); the same was true upon $\frac{1}{4}$ strength. On those regimes the rats grew at usual rates. Over periods of two weeks, therefore, no deleterious effects were evident from drinking diluted sea water, and, providing it was dilute enough, full food intake and body weight prevailed.

Ingestion of sodium chloride solutions. When solutions of sodium chloride were substituted for sea water, the same results were obtained. The same individuals studied above were given solutions 0.75 M, 0.50 M, 0.37 M, 0.25 M and 0.12 M to drink. The first two concentrations were in general drunk sparingly, while the last three were taken in amounts greater than distilled water (fig. 2). Thus, the amounts of 0.25 M solution that were drunk were sufficient to furnish the usual available water and in addition to carry away in urine the extra salts ingested.

Evidently the factors which guide the ingestion are in tune with the limited concentrations in excretion, as was evident in the experiments of Gamble *et al.* (1929, 1934) in which the salts were mixed in the food. In those experiments the amounts of water voluntarily ingested by rats were such as to render the total particulate concentrations of diverse electrolytes in urine equivalent to one another, about 1.0 osmolar (for univalent salts, 0.50 M).

Ingestion of ammonium lactate solutions. For contrast with solutions of salts that are excreted unchanged, a salt that is metabolized was chosen. Ammonium is converted to urea, and lactate is oxidized to carbon dioxide. A 0.36 M solution of ammonium lactate was provided in the drinking burettes, in place of tap water, for periods of 12 days. The consumption was uniform upon successive days and amounted to 61 per cent of the previous rate of ingestion of fresh water. Apparently the 3 rats tested maintained themselves upon this solution, drinking sufficiently for their metabolisms, in spite of the sour taste.

Water privation. Control tests for all the above experiments were furnished when no water was allowed to the rats. Food was then consumed in diminishing amounts until, after 4 days, less than ten per cent of the usual amounts were eaten (fig. 1). Weight was lost; urine was scanty and highly concentrated.

Evidently the aversion to food helped to preserve the proportions in the body between water and solutes, whereas ingestion of more food would have acted to make the shortage of water more acute. It is well known that the rat refuses food whenever some essential constituent of its intake is lacking; this is the way in which curves of body weight as regularly employed in dietary studies are able to manifest the inadequacy of a diet. Water is one such constituent.

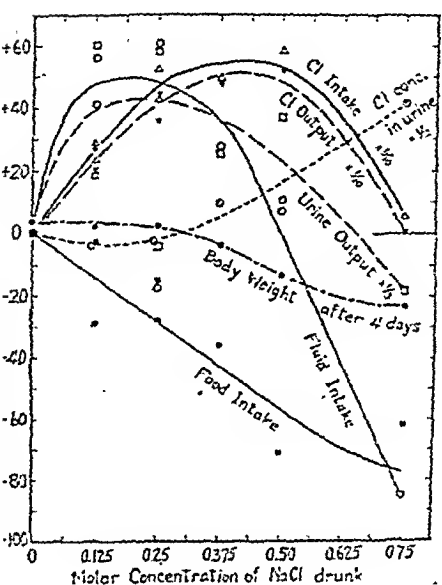


Fig. 2

Fig. 2. Increments of various exchanges *etc.*, in rats maintained for 4 days on drink containing diverse concentrations of sodium chloride. Exchanges were measured in daily periods and averaged, each point representing one individual. Dry milk was always available *ad libitum*.

Fig. 3. Increments of body weight, and exchanges in per cent of body weight per day, in rats first deprived of drink, O, for one day (5 tests each) or for two days (3 tests each) and then presented with various concentrations of sodium chloride (M/1, M/2, M/4) or fresh water, W, to drink *ad libitum*. Dry food was always available *ad libitum*.

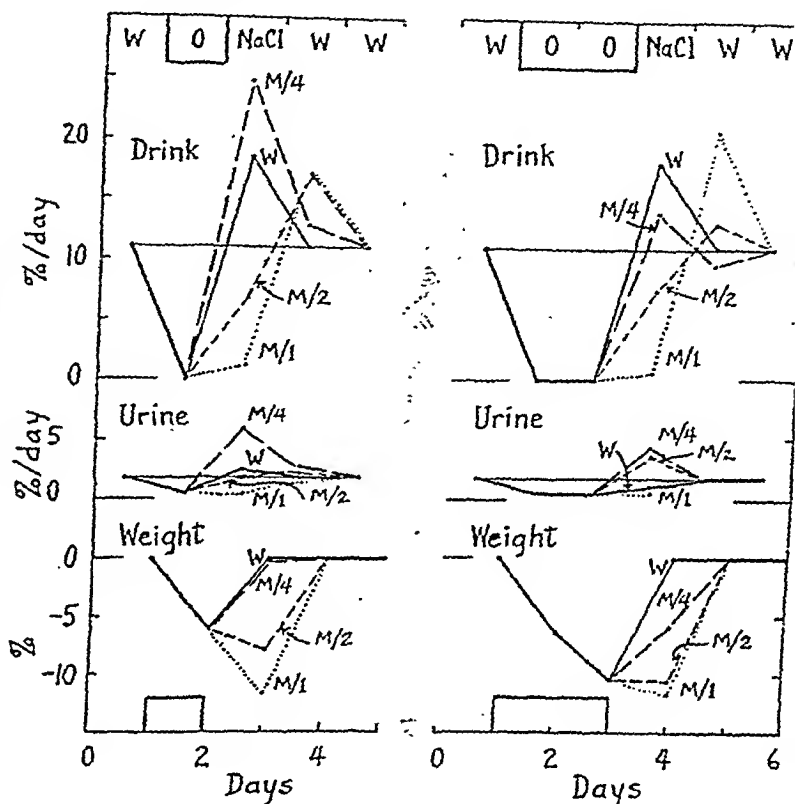


Fig. 3

When food as well as water was denied the rat, loss of weight proceeded at the same rates as when food was allowed. What small food intakes occurred in absence of water were evidently such as to add nothing to the net content of the body so long as water was not available.

When water was allowed but food was denied, the same picture of weight loss ensued. This loss occurred in accordance with the fact that water intakes were after the first two days only one-fourth to one-twentieth of the usual ones.

So far as data are now available, privation of some one constituent of the diet

leads to as much loss of weight as does the privation of everything. What else the experimenter does not deny the animal, the animal itself denies its body. And even the moderate amounts taken by the animal are scarcely or not at all retained.

Survival. Fifteen male rats (mean initial weight 179 grams), kept in air temperatures of 22° to 28°C, survived without water for 6 to 15 days. Two of 16 additional rats given water upon the sixth day, failed to recover ultimately, though able to drink and eat for one to three days longer before death. The 15 first rats lost 46 per cent of their initial body weights at death.

Fifteen other rats given sea water to drink survived for 7 to 26 days. The deficit of body weight at death was the same as above, 46 per cent. However, the mean survival time was increased from 10.2 days to 13.7 days; this increase was probably significant ($\Delta 3.5$ days ± 1.5 standard error of the difference). All rats of the two groups of 15 each were not studied simultaneously; this fact increased the variability of conditions. However, representatives of each group were paired, and were weighed daily in order that any chance access to water would be detected. Ingestion of sea water appeared to diminish the rate of weight loss, and in 11 of the 15 pairs of individuals survival was aided by it. Heller (1932) showed that survival was not indefinite when equivalent solutions of various salts were drunk by rats.

Rats deprived of both food and water survived 7 days or more. Deprived of food alone, they survived no longer, and lost just as much weight. This result contrasts with that for larger animals in which stores of food outlast stores of water.

Salt solutions after water privation. Solutions of sodium chloride may be substituted for water in periods of recovery from previous dehydration. For these tests rats were deprived of water for either one or two days, then presented with the solutions. Whereas the rats that were allowed water invariably drank, within one hour, amounts nearly equal to the body weights they had lost during privation, those given 1.0 M NaCl drank practically nothing during the first hour. They repeatedly tasted the solution at the burette and refused it. In the subsequent 24 hours, however, about 1 per cent of the body weight was consumed (fig. 3).

When given 0.5 M NaCl after the privation of water, rats drank very little during the first hour; but during 24 hours consumed more than half the volume of tap water ordinarily consumed in one day.

When given 0.25 M NaCl at the close of water privation, the rats took more than they took of water itself. By ingesting double the usual volume, they thus paid off the debt of body water and at the same time were able to eliminate the salt taken in this drink.

Sea waters in series. In another set of experiments rats always had drink available but were made to shift successively from one kind of drinking solution to another. One object was to see whether by starting with a diluted drink, rats could later utilize whole sea water to greater advantage. In none of five tests

ingestion and subsequent excretion of salts, slower than they lost it by evaporation.

3. Half sea water, or equivalent solution of sodium chloride, allowed indefinite maintenance of body weight in rats, with augmented turnovers of water and salt.

4. Maintenance of water content was not favored by progressive increase in the salt concentration of drinking water.

5. Recovery from dehydration as a result of previous water privation did not occur when sea water or equivalent concentration of sodium chloride was allowed. When fresh water was given the corresponding recovery required several days' time.

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THE BASAL METABOLISM OF MID-WESTERN COLLEGE WOMEN¹

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As part of a regional study of the nutritional status of college women, the basal metabolic rates of women students living in 5 mid-western states have been investigated. It was desired to determine the normal basal metabolism for these age-groups and, as a range of latitude was represented in the cooperating states, to note if geographical differences were evident.

Present basal metabolism standards for women are generally believed to be too high, particularly through late adolescence and early maturity (19). It is customary to use adult standards for these ages but this practice has been questioned as evidences of growth are often apparent during these years (20, 24). Harris and Benedict (13) found the limits for various stages of development were indefinite which fact further complicates the establishment of standards. They also suggested that measures of variability are quite as important as average values.

In a study of girls, Stark (19) observed the adult type of metabolism becoming apparent from 17 to 21 years of age. In later work (20) she suggested the ages of 16 to 21 years as a border line period during which some growth might be expected to occur. Benedict and Hendry (3) reported a distinct but slight decrease in the basal rate of adults, the average amount per year for women being 2.29 Calories per 24 hours. With young subjects, particularly girls of 5 to 17 years, the decrease is much greater according to Boothby and Sandiford (5).

A great range in the basal metabolism of Connecticut College women made Rogers (18) doubt if the subjects were always in a basal state at the time of a test, even though they appeared to be, in so far as outward indications were concerned.

According to Benedict (2), women show more day-to-day variability in the basal rate than men. He attributed this to the effects of the menstrual cycle and suggested that these individual differences were more or less equalized when large groups of subjects were used.

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Jenkins (15) advocated the determination of the mean basal metabolic rate of a series of normal individuals for each particular region. This is in accord with the work of several investigators (7, 16, 17, 21, 23) who find geographical location an important factor in basal metabolism. Du Bois (8), however, suggested that things other than climate may account for some of these differences.

Results of studies of the effects of season upon basal metabolism do not agree. Gustafson and Benedict (11) observed that sunshine and humidity, as well as temperature, were involved. They noted a tendency to low levels in winter and high ones in spring and summer. Tilt (22) found no seasonal change in the basal rate while Hafkesbring and Collett (12) obtained higher results in cold than in warm weather.

Before better standards can be established it is generally believed (8, 13, 19) that many studies must be made including large numbers of normal persons of all ages and types and representing many different localities. The present studies are submitted as a contribution to this end.

PROCEDURE. The 5 co-operating states were Iowa, Kansas, Minnesota, Ohio and Oklahoma. With the exception of Oklahoma, in which the subjects were studied for 3 years, the basal determinations were made over a 5-year period or longer. The subjects were regarded as normal in health as determined by general appearance and freedom from illness. No thyroid disturbances were apparent. The young women were regarded as typical of the student population in each state. While the ages ranged from 17 to 24 years inclusive, the majority of subjects in each state fell into the 18- to 22-year groups.

The tests were made under the usual standard conditions (8). A closed-circuit portable respiration apparatus of the Roth-Benedict type was used. The machines were carefully checked for accuracy. Operators were skillful in handling apparatus and subjects, although some were apparently more efficient than others (6). The methods used were uniform for the co-operating states and were those generally accepted as reliable. No determinations were made during the menstrual period or at any other time of known excitement or strain.

Duplicate 8-minute tests were made on a subject on any given day with a 2- to 3-minute period of rest between. With the exception of a few early cases which were tested on one day only, a total of 4 tests was obtained for each subject, two on each of 2 days which were scheduled as close together as possible. The mean of the 4 tests was used as one determination of the basal metabolic rate. In many instances, but not always, the 4 tests agreed within the conventional 5 per cent formerly regarded as essential.

A total of 1179 determinations was made on 576 different subjects. These were distributed as follows: Iowa—264 determinations, 154 subjects; Kansas—379 determinations, 158 subjects; Minnesota—283 determinations, 114 subjects; Ohio—172 determinations, 114 subjects; Oklahoma—81 determinations, 36 subjects. Many of the young women were studied each year they were in college, so appear in different age-groups. This accounts for the fact that the individuals are fewer than the number of determinations.

RESULTS AND DISCUSSION. Preliminary treatment of the data indicated that

statistically significant differences existed between states. Consequently the findings for each state are reported separately (table 1). Because of unequal numbers and, in some cases, too few subjects in the different age-groups, and also because significant differences appeared to exist between ages in some of the

TABLE 1

Mean basal metabolism of mid-western college women

AGE	NO.	CAL./ SQ.M./HR.	STANDARD ERROR	5 PER CENT FIDUCIAL LIMITS	AGE	NO.	CAL./ SQ.M./HR.	STANDARD ERROR	5 PER CENT FIDUCIAL LIMITS
Iowa					Kansas				
17	16	35.9	0.562	34.7-37.1	17	38	35.0	0.504	34.0-36.0
18	31	35.1	0.379	34.3-35.9	18	58	33.6	0.336	32.9-34.3
19	48	35.0	0.368	34.3-35.7	19	77	33.2	0.317	32.6-33.9
20	52	35.1	0.328	34.4-35.8	20	89	32.8	0.265	32.3-33.3
21	50	34.6	0.331	34.0-35.3	21	56	33.1	0.283	32.5-33.7
22	38	34.6	0.316	34.0-35.3	22	37	32.7	0.377	32.0-33.5
23	18	35.0	0.655	33.6-36.4	23	13	32.9	0.462	31.9-33.9
24	11	34.1	0.665	32.6-35.4	24	11	32.4	1.044	30.1-34.7
Total....	264					379			
Minnesota					Ohio				
18	34	35.7	0.413	34.9-36.6	17	34	36.4	0.381	35.6-37.2
19	76	34.9	0.257	34.4-35.4	18	40	33.8	0.391	33.0-34.6
20	65	33.9	0.269	33.4-34.5	19	35	34.0	0.326	33.3-34.7
21	48	33.5	0.356	32.7-34.2	20	22	34.8	0.520	33.7-35.9
22	37	33.5	0.416	32.7-34.4	21	32	34.0	0.430	33.1-34.9
23	15	33.3	0.416	32.4-34.2	22	9	34.6	0.787	32.8-36.4
24	12	32.2	0.526	31.0-33.2					
Total....	283					172			
Oklahoma									
17	8	34.4	0.420	33.4-35.4					
18	19	34.0	0.617	32.7-35.3					
19	25	33.6	0.460	32.7-34.5					
20	20	32.3	0.408	31.4-33.2					
21	9	31.1	0.674	29.5-32.7					
Total....	81								

younger groups, it was not possible to consider the entire number of students within a state as a homogeneous lot. This made it impossible to determine a mean basal rate for each state which included all subjects. Actually this could be done only for the ages of 20 to 22 years inclusive, and for 4 states. Analysis of variance showed the Oklahoma subjects were significantly different at 20 years, and after this age the numbers were too few to give reliable results. Therefore

no general mean was computed for this state, although it appears that the basal rates are undoubtedly as low or lower than those of any other state studied (table 1). The other 4 states are ranked in descending order as to their basal metabolic rates (table 2). An analysis of variance based on these limited data showed the differences between states for these ages were no longer as great as they had been when all the age-groups were used ($P < 0.08$). Even so, with a figure so close to significance as this, it still seemed advisable to consider the states separately.

In table 2 the states are again ranked, but in ascending order, according to the mean temperatures for the years of 1934-1938 inclusive, which cover the greater part of this study. Assuming, as has been suggested by some workers, that basal metabolism is inversely proportional to temperature, the mean basal rate in the different states might then be expected to place in the same relative position as for temperature but in inverse order. In accord with this idea, the subjects from the warmer states, i.e., Kansas, definitely (table 2), and Oklahoma,

TABLE 2

Basal metabolic rates of the co-operating states compared with the mean temperatures (25)

STATE	SUBJECTS 20-22 YRS., INC.	MEAN	STATE	MEAN TEMPERATURES 1934-1938, INC.
	<i>no.</i>	<i>Cal./sq.m./hr.</i>		<i>°F.</i>
Iowa.....	140	35.8	Minnesota.....	41.6
Ohio.....	63	34.4	Iowa.....	49.5
Minnesota.....	146	33.7	Ohio.....	52.0
Kansas.....	182	32.9	Kansas.....	56.6
			Oklahoma.....	61.7

apparently (table 1), had slightly lower basal rates than those from the colder regions. However, Minnesota, the coldest state, did not have the highest rate as might have been expected.

The possibility of differences in thyroid activity in different localities probably should be considered as a possible explanation for these discrepancies. Minnesota and portions of Ohio are part of a region naturally deficient in iodine. Lack of iodine is known to cause thyroid changes which result in lowered basal metabolism. While the use of iodized salt was general in these regions during the period of the study, it is possible that there may have been some deficiency in some of these subjects. This may explain to some extent the somewhat low rate of Minnesota students which was evident in spite of a cold climate. Stark (19) has reported for a somewhat younger group of 47 Wisconsin girls (19 to 20 yrs. old) a similar basal rate of 33.5 Calories per square meter per hour (table 2). Wisconsin also has a cold climate and is in the iodine-deficient region so a similarity in basal rates would be expected and apparently existed for these two states.

The range in altitude, 703 to 1002 ft., exhibited by the 5 states (table 3) is slight. The difference of 299 ft. is too small to have affected results appreciably according to Iliff et al. (14).

For all states the means of the first year's determinations (table 1) were higher than those for succeeding years. For Kansas and Ohio these differences were significant (Kansas, 17 and 18 years, $P < 0.01$; Ohio, 17 and 18 years, $P < 0.001$). From the standpoint of age a higher rate might be expected, for the subjects at this time were younger and in the border line period but also, with a few exceptions, these were the means of first determinations so it is unlikely that age is the sole explanation of this higher rate although it is probably the chief one. According to Du Bois and Chambers (9) and Stark (20) first observations are usually high, due to unavoidable nervous tension.

The mean results for all ages in all states covered by this study (table 1) are lower than those of Boothby, Berkson and Dunn (4) for women of corresponding ages representing different sections of the country but tested at the Mayo Clinic. In part, at least, this is due to the fact that these workers used only their first single, satisfactory test assuming that their results would thus be more suitable for clinical standards. These means are also all lower than the Du Bois stand-

TABLE 3
Altitudes (10) represented in study

STATE	CITY	ALTITUDE <i>ft.</i>
Kansas.....	Manhattan	1002
Iowa.....	Ames	926
Oklahoma.....	Stillwater	870
Ohio.....	Columbus	744
Minnesota.....	Minneapolis	703

ards as modified by Boothby and Sandiford (5) who recommend 36.9 Calories per square meter per hour for women of 20 to 24 years. This figure is frequently used for computing the normality of basal tests.

Body temperature has been accepted as a factor affecting the basal rate, the metabolism supposedly rising with the temperature. According to Barnes (1), the temperatures of women are particularly variable and many subnormal values are observed. He suggested an average temperature of about 98.0°F. for women but found it varied with the menstrual cycle from a low of 97.5°F. at the time of ovulation to a peak of near 98.5°F. shortly before the beginning of the menstrual flow.

Body temperatures recorded for this study showed considerable individual variation but practically all were below the 98.6°F. regarded as normal. The fact that the temperatures were taken early in the morning would account to some extent for this low level. It was not possible to know when ovulation was a factor but it undoubtedly was occurring in some cases. The majority of these subjects registered temperatures between 97.5° and 98.0°F. and the mean for all subjects from the 5 states was 97.9°F. This agrees well with Barnes' mean (1) of 98.0°F. The means for the individual states fell close together, Iowa being lowest with 97.7°F. However, in spite of a low body temperature,

Iowa subjects showed the highest basal rate (tables 1, 2). Little correlation was found between basal metabolism and body temperature in this study although it has been suggested (1) that physicians may use a low temperature as indicative of need for thyroid therapy.

Basal rates also gave low correlations with respiration and but slightly higher ones with pulse rates. Stark (20) has suggested the existence of a rough parallelism of pulse with the metabolic rate but according to Barnes (1) pulse is not a reliable index of basal metabolism. The latter would seem to be true of the results of this study in most instances.

As originally planned, the basal metabolic rates of freshman women were to be followed by means of yearly tests during their stay in college. This was difficult to carry through for obvious reasons and subject losses were great. Only 193 of the 576 different subjects for the 5 states were studied as often as once each year for 3 or more years. Actually 9 different subjects were followed for 5 years, 98 for 4 years, and 86 others for 3 years.

No striking trends were evident. Of the entire group, 101 or 52.3 per cent were highest at their first determination but only 51 individuals or 26 per cent showed a more or less steady decline through the time they were studied. Sixteen subjects or 8.2 per cent remained constant (± 1 Calorie) for the 3 or more years of the experiment. The remainder were likely to be high one year and low the next or vice versa. As a rule, intra-individual variations in these yearly tests were sufficiently great to mask any definite decreases in the metabolic rates that could be attributed to age. Certainly no marked changes occurred in the basal rate during these years although the metabolism was by no means static.

SUMMARY

Basal metabolism determinations have been made on 1179 college women from 5 mid-western states. These represented 576 different individuals ranging in age from 17 to 24 years, inclusive.

The basal rates for the different states were significantly different. Something more than temperature in the geographical regions represented is needed to explain the differences in basal rates although it would seem to have some influence. In general, the rates were lower in warmer climates.

Body temperatures were lower than the accepted standards for subjects in all the states represented in this study.

Little correlation was evident for basal metabolism with body temperatures, respiration, or pulse rates.

First tests tended to be high.

Yearly tests on the same subject over a 3- to 5-year period indicated that intra-individual variations tended to mask any changes from year to year due to age.

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INDICATOR YELLOW¹

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The decomposition of visual purple results in the formation of a yellow color. After years of doubt it has been finally established that a yellow substance is formed but there is a question whether there are one or more yellow substances.

Kühne (1) observed that the retina or a solution of visual purple upon exposure to light before becoming colorless turned orange, deep yellow and light yellow in color. He termed the yellow substance visual yellow or xanthopsin. Following this observation many papers (2) were published giving evidence for and against the formation of visual yellow. Unknown experimental conditions apparently varied the color.

In 1929 Naskashima (3) noticed that visual yellow changed from a deep yellow in acid solution to a light yellow in alkaline solution. Chase (4) showed by spectral absorption curves of bleached visual purple solutions that a yellow substance which was affected by different hydrogen ion concentrations was formed. Wald (5) extracted a yellow substance which he called retinene and which he believed to be a carotenoid from its color reaction with antimony trichloride.

Lythgoe (2) named the yellow substance which changed color, indicator yellow. He and Quilliam (6) found that spectroscopically visual purple formed an orange colored substance which he termed transient orange. Visual yellow was considered to be a mixture of transient orange and indicator yellow. Indicator yellow was thought to be derived directly from visual purple or through the intermediate transient orange. In contrast to the visual yellow or retinene of Wald neither of these two substances according to Lythgoe were extractable from aqueous digitonin solutions with petroleum ether.

Although Granit (7) believed that retinene and indicator yellow are two different substances, Hecht (8) recently gave the opinion that visual purple breaks down into a protein and a yellow substance. The colored substance was considered to be visual yellow or retinene, the depth of color of which varies with the hydrogen ion concentration.

This investigation is concerned with the characteristics of a decomposition product of visual purple which has the properties of indicator yellow and not those of visual yellow (retinene).

PREPARATION. The retinas were obtained from bovine eyes at the killing floor of the slaughter house. They were immediately dropped into ice-cold alcohol.

About 10,000 retinas were extracted with 20 liters of an aqueous solution of 70 per cent ethyl alcohol. The alcoholic extract was shaken with 2 liters of

¹ This investigation has been supported by a grant from the John and Mary Markle Foundation.

petroleum ether (b.p. 30–60°C.). The petroleum ether fraction was separated and the solvent evaporated to dryness by vacuum. The residue weighing 38 grams was dissolved in 100 cc. of alcohol and 4 grams of potassium hydroxide dissolved in 25 cc. of water were added. The solution was allowed to stand for two hours at 26°C. and was then extracted with petroleum ether to remove the unsaponifiable material and visual yellow. The alcoholic solution was diluted with 75 cc. of distilled water, made acid with hydrochloric acid and then extracted with petroleum ether to remove the fatty acid fraction. The alcoholic solution was diluted with 75 cc. of distilled water and then extracted with ethyl ether. The ether soluble fraction possesses a deep yellow color. After evaporation of the ether the residue was golden brown in color. The treatment with aqueous alcohol and ether was repeated for purification of the indicator yellow. The yield from 10,000 retinas was about 150 mgm.

The first petroleum ether fraction contained pro-indicator yellow and visual yellow. These two pigments could not be separated. By the alkaline hydrolysis, indicator yellow was liberated. The petroleum ether extracted the visual yellow from the alcoholic solution leaving the indicator yellow to be extracted with ethyl ether.

A yellow substance possessing the indicator yellow reaction to acids was separated from visual yellow by another method which was unsuitable for chemical procedures leading to isolation of individual compounds. After the bacillary layer of the retina was bleached to a yellow color upon exposure to light, it was extracted with a 1 per cent aqueous solution of sodium glycocholate. Upon acidification of the clear yellow extract, a yellow color precipitate formed in a colorless solution. Apparently a visual yellow protein was precipitated. No test for protein was made. The remaining bacillary layer of the retina insoluble in the glycocholate solution was extracted with a 1 per cent aqueous solution of digitonin. The clear extract upon acidification gave no precipitate and turned the deep yellow of indicator yellow. The reverse process was tried. The bleached bacillary layer of the retina was extracted with digitonin solution. The extract turned yellow upon acidification. The retinal residue insoluble in the digitonin solution was extracted with a 1 per cent solution of glycocholate. The clear yellow extract upon acidification with hydrochloric acid gave a yellow precipitate and a colorless solution.

PROPERTIES. Indicator yellow was a dark brown viscous solid which was slightly soluble in petroleum ether and soluble in alcohol, ether benzene, acetone and chloroform. It was soluble in 70 per cent aqueous alcohol from which it was extractable with ether or chloroform but not petroleum ether. In contrast visual yellow was extractable with petroleum ether. Indicator yellow in aqueous digitonin solutions of bleached visual purple was not extractable with ether. It was extractable from one per cent aqueous alcoholic solution of sodium hydroxide by ether or chloroform but not by petroleum ether.

In acid alcohol indicator yellow gave a feeble pale green fluorescence upon exposure to ultra-violet light. In alkaline alcohol it produced a remarkably strong yellow green fluorescence.

With antimony trichloride in chloroform it gave a pale pink color which has no distinct spectral absorption in the visible spectrum. The $E_{1\text{ cm.}}^{1\text{ per cent}}$ of the spectral absorption maximum at 360 mu in absolute alcohol at pH 8.0 was 212 and at 444 mu at pH 5.2 was 163.

The reaction with Girard T. reagent suggested that indicator yellow possesses a carbonyl group which is formed by the hydrolysis of pro-indicator yellow. Visual yellow and pro-indicator yellow showed no reaction with this reagent.

Barium or copper acetate gave no precipitate with an alcoholic solution.

By micro-combustion a sample which was the purest obtainable gave an average of 52.96 per cent carbon, 8.32 per cent hydrogen, 1.99 per cent nitrogen, 0.48 per cent phosphorus, and 6.76 per cent ash. No sulfur was present.

DISCUSSION. Of the various methods for the isolation of indicator yellow which were tried the most successful procedure is given. The retinas were processed in batches of 2000 until the material of about 20,000 to 40,000 eyes was accumulated. As far as can be determined by qualitative tests and by spectroscopic absorption the indicator yellow which was isolated is identical with that found in bleached visual purple solution obtained from bovine retinas.

All attempts to separate pro-indicator yellow from visual yellow have failed. The solubilities are similar and chromatographic materials tend toward destruction of the substances. Because pro-indicator yellow and visual yellow are both extractable from aqueous alcoholic solutions of bleached visual purple by petroleum ether and chloroform and indicator yellow by chloroform and not by petroleum ether, the confusion over properties of the yellow substances is explained. However, the digitonin and glycocholate which are used in aqueous solutions to dissolve visual purple greatly modify solubilities by the formation of new complex compounds with the lipids.

The properties of pro-indicator yellow suggest that it is a fatty acid ester without a free carbonyl group and it is a part of visual purple molecule. Apparently indicator yellow possesses a free carbonyl group and has few if any carotenoid properties. The percentage of nitrogen and phosphorus and other properties indicates that it is not a simple phospholipid.

The part that indicator yellow plays in the vision is unknown. Since many investigators find it present in bleached visual purple solutions when the test for change of color is made either chemically or spectrographically it seems that the visual process is more than a splitting off of visual yellow (retinene) from a protein and that indicator yellow is also formed. Indicator yellow according to its composition and color reaction is not a conjugated visual yellow or a derivative of visual yellow. Its antimony trichloride reaction is not characteristic for a carotenoid.

The names, visual yellow for retinene and indicator yellow, should be used until the chemical structures are determined. Retinene by chemical terminology is a hydrocarbon but no chemical proof has been given. Its properties suggest that it is a carotenoid but the chemical evidence is decidedly meager.

SUMMARY

A yellow lipid, provisionally named pro-indicator yellow, was extracted from bovine retina. After alkaline hydrolysis a fatty acid and indicator yellow were isolated. A study of the properties of indicator yellow shows that it is remarkably different from visual yellow. Its spectral absorption curve shifts towards the longer wave-lengths with the increase of acidity of the solutions. Indicator yellow is one of the substances which is found after visual purple is affected by light.

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THE EFFECT OF ADRENALECTOMY ON THE ABSORPTION OF THE SHORT CHAIN FATTY ACIDS AND THEIR TRIGLYCERIDES

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It has been demonstrated earlier by Verzar and Laszt (1935) and later by Bavetta et al. (1941) and Bavetta and Deuel (1942) that a definite inhibition of fat absorption results in rats from the total ablation of the adrenals. Bavetta et al. (1941) also demonstrated that this depression in fat absorption by adrenalectomized animals could be restored to normal by the administration of a potent cortical extract. These findings have been opposed by several investigators, notably Barnes, Miller and Burr (1941), who observed that adrenalectomized rats maintained in a good state of health by the administration of salt solution absorbed fat at the same rate as normal controls. At that time we felt that the failure of Barnes et al. (1942) to observe any significant difference in fat absorption in their adrenalectomized rats was due to the fact that they employed much larger and older rats. It was pointed out that cortical deficiency is much more critical in younger animals.

In our previous experiments (1942) it was noted that the absorption of tributyrin and of sodium butyrate was unaffected by adrenalectomy. This suggested that the absorption of the lower and more soluble fatty acids is probably not dependent on adrenal activity. As a result of this observation, it seemed desirable to undertake a systematic study of the relationship of solubility to absorption.

METHODS. The procedure employed for the study of absorption of neutral fats was similar to that reported earlier (1941), while the method used for the determination of volatile acids, when fed as soaps, was the same as that described by Deuel et al. (1941). In all cases recovery experiments were first conducted in order to ascertain the efficiency of the method employed. The rats used in these recovery experiments were fasted a similar period of 24 hours before the recovery tests were performed.

Capric and caprylic acids, when fed as such, were recovered by washing out the gut with 80 cc. of absolute isopropyl alcohol instead of diethyl ether as employed by Deuel et al. (1941). The amount of the acid was determined by direct titration. In all cases a subtraction was made for values found in fasting controls. The recovery values obtained were 94.9 per cent for capric acid; 90.4 per cent for the tricaprylin; 94.9 per cent for the caprylic acid; 95.5 per cent for the sodium caprylate; 88.1 per cent for the tricaproin; and 90.2 per cent for the sodium caproate. In all cases at least 10 animals were used for each series of tests.

In all experiments the absorption period was 3 hours except with capric acid where an 8 hour absorption period was used.

The rats used in these tests were female rats weighing approximately 120 grams which had been maintained on our regular stock diet. The triglycerides and fatty acids employed were all Eastman products, the purity of which was determined by their saponification numbers. Because of the marked laxative effect of tricaprion (Deuel et al., 1940), it was not tested here.

RESULTS. The average results are summarized in table 1 which also include for comparison the mean values previously reported from this laboratory.

It was found that the absorption of tricaproin and of sodium caproate was not depressed by adrenalectomy. The absorption of tricaprylin also shows no appreciable difference between normal and operated animals. However, there is a significant depression in the resorption of caprylic acid. This decrease was, to some extent, corrected by feeding this acid as the water-soluble sodium caprylate. The absorption of capric acid was also very significantly depressed by the extirpation of the adrenal glands. Thus, the cortical influence on fat absorption which

TABLE 1

Absorption of capric acid, tricaprylin, caprylic acid, sodium caprylate, tricaprion and sodium caproate by normal (N) and adrenalectomized (A) female rats

FED	ABSORPTION PERIOD	NUMBER OF TESTS		BODY WEIGHT IN GRAMS		ABSORPTION IN MGM. PER 100 SQ. CM. PER HOUR*		M.D.: S.E.M.D.†	TITRATION IN CC. OF 0.1 N NaOH*		M.D.: S.E.M.D.	ABSORPTION EARLIER‡
		N	A	N	A	N	A		N	A		N
	hours											
Capric acid	8	13	10	96	116	18.2±1.0	12.8±0.5	4.32				22.6±2.1
Tricaprylin	3	13	10	110	130	47.1±3.2	43.8±3.5	0.70	3.83±1.2	6.3±1.0	1.60	45.9±4.1
Caprylic acid	3	11	11	122	142	42.5±1.2	28.5±2.1	5.78				46.0±1.7
Sodium caprylate	3	12	10	110	130	50.1±1.3	43.2±2.0	2.89				
Tricaproin	3	13	11	132	134	52.4±1.8	51.5±4.4		1.4 ±0.5	3.7±0.8	2.65	54.5±1.5
Sodium caproate	3	12	12	106	140	43.2±3.0	45.4±1.5					38.0±1.7
Sodium butyrate§	1.5	16	14	122	103	45.0±2.6	42.8±2.3					
Tributylin §	3	11	16	108	117	69.1±3.7	65.8±1.8		0.6 ±0.3	0.5±0.1		

* Including Standard Error of Mean.

† Mean Difference: Standard Error of Mean Difference. When this ratio exceeds 3, the results are considered significant.

‡ Results from Deuel and Hallman (1940) and Deuel et al. (1941).

§ Bavetta and Deuel (1942).

seems to start with more soluble caprylic acid is very decidedly evident with capric acid.

DISCUSSION. The present experiments support our earlier conclusions that whereas the natural fats require the presence of the adrenal cortex for their efficient absorption, the short-chain fatty acids which are more water soluble and also their triglycerides are absorbed as rapidly in the adrenalectomized as in the normal rat. In the present tests it is shown that the absorption of tricaproin or sodium caproate is not influenced by adrenalectomy as was noted earlier for tributyrin and sodium butyrate.

One may regard tricaprylin as an intermediate member between those triglycerides where the fatty acids are water soluble and those where they are practically insoluble. While butyric acid is infinitely soluble in water, caproic acid dissolves to the extent of 0.89 part, caprylic to 0.079 part and capric to only 0.0034 part per 100 cc. of water.

The need for some cortical factor begins to manifest itself with caprylic acid and becomes very acute in the case of capric acid. It is to be noted that the absorption of tricaprylin is not significantly altered by adrenalectomy. However, a somewhat greater accumulation of fatty acids was found in the operated group as compared with the normal controls. While this difference is not significant, it does, however, show that the caprylic acid liberated as a result of hydrolysis is absorbed at a slightly lower rate by adrenalectomized rats. This differential rate is definitely proved and magnified by the results obtained in the absorption experiments where caprylic acid itself was fed. This depression was corrected to an appreciable extent by feeding the more soluble sodium caprylate. One must conclude from such data that in the case of tricaprylin the caprylic acid formed as a result of its hydrolysis in the intestine becomes available at a slow enough rate so as to be absorbed equally well by both groups of rats. It is thus possible that the adrenals have a relative rather than absolute influence on the permeability of the intestinal epithelial cells. The caprylic acid thus provided would not overtax the capacity of the intestinal epithelium of either the operated or normal animals to absorb the liberated fatty acids. However, flooding the absorption mechanism with an oversupply of caprylic acid would bring out the difference in powers of absorption between normal and adrenalectomized groups.

While the absorption of caprylic acid, when fed as such, was lowered 33 per cent, that of capric acid was decreased to no greater extent (29 per cent). In the latter experiments, the rate of absorption was so slow that the experiments were continued for 8 hours to obtain accurate results. The results of the absorption on normal rats agree closely with those reported earlier.

SUMMARY

There was no significant depression in the absorption of tricaproin, sodium caproate, and tricaprylin in adrenalectomized animals as compared with normals. The first indication of any inhibitory effect resulting from adrenalectomy occurs with caprylic acid and is also evident with capric acid. This indicates that the adrenal glands play an active rôle in the absorption of the longer chain fatty acids. However, the absorption of lower acids is not dependent on adrenal function. Differences in the absorption rates of the longer chain fatty acids by normal and adrenalectomized animals are probably due to the capacity of normal animals to remove fatty acids from intestine at a much faster rate.

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A STUDY WITH RADIOACTIVE ISOTOPES OF THE PERMEABILITY OF THE BLOOD-CEREBROSPINAL FLUID BARRIER TO IONS¹

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The mode of formation of C.S.F.² is a problem of fundamental importance in the fields of physiology and biochemistry. Flexner (3) states that the key to the problem is the rôle played by the tissue membranes separating blood plasma and C.S.F. in the distribution of ionic and molecular species between these two fluids. The question is, do the endothelial or ependymal elements of the barrier between the blood and C.S.F. actually perform work in the formation of C.S.F., that is, do they play an active, secretory rôle, or is the distribution of substances between the fluid and the plasma maintained by passive diffusion such as would occur across an inert membrane impermeable to colloidal particles?

The literature dealing with this problem has been extensively reviewed in articles (3, 4, 5) and monographs (6, 7) and it would only be repetitious to consider it here.

A somewhat different hypothesis of the mechanism of the formation of C.S.F. has recently been proposed by Wallace and Brodie (8), who suggested that the dissolved substances first pass from the blood plasma into the extracellular fluid spaces of the brain and cord and from these into the C.S.F. Equilibrium, when it is established, is not between the plasma and C.S.F., but between the extracellular fluid of the central nervous system and the C.S.F. According to these authors, the sequence of passage of dissolved substances is from the plasma into a pericapillary and perineuronal space constituting the extracellular tissue space. In this first passage they must cross a barrier which offers some selective hindrance to their course. From the extracellular spaces, the dissolved substances enter the perivascular spaces and thence pass into the subarachnoid fluid. The choroid plexus, heretofore believed to be the major site of the formation of C.S.F., is relegated to a minor rôle in determining the composition of the C.S.F.

The experimental evidence for the foregoing hypothesis is that the extracellular fluid space of the central nervous system is distinct in character from that of the extracellular space of tissues elsewhere. Passage of dissolved substances into the

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² To save space C.S.F. will be used as an abbreviation for cerebrospinal fluid.

extracellular space of tissue is rapid, while into the extracellular space of the central nervous system it is slow.

The recent developments in the use of radioactive isotopes, artificially produced by the cyclotron, afford a convenient and valuable method of studying the properties of the barrier between the blood and the C.S.F. for the normal ion constituents of the body.³ The isotope method is admirably suited to the study of problems of permeability. By the use of labeled ions, it is possible to discern a transfer or exchange in a system in which there are no net changes in concentration. Such labeled ions may be distinguished from the ions already present in the body fluids by the radiation which is emitted when the unstable isotopes disintegrate. Otherwise there is no chemical or physiological difference.

The authors have undertaken the study of the formation of the C.S.F. by means of such labeled ions. The aim of the investigation was to measure the rates at which ions present in the blood plasma appeared in the spinal fluid. By the use of salt solutions labeled with the isotopes already mentioned, it was possible to distinguish the administered ions from those normally present in the body. As was to be anticipated, it was found that the permeation of ions from the blood stream into the C.S.F. is generally a slow process; many hours are required for the labeled ions to reach the normal ratios found by chemical analysis.

The results of this study favor the secretion theory of formation of the C.S.F.

EXPERIMENTAL METHODS. Large, normal dogs weighing between 15 and 30 kgm. were used in these experiments. After anesthetization with intravenous sodium pentobarbital in doses of 30 mgm. per kgm. of body weight, a cisternal puncture was aseptically performed with a no. 19 gauge spinal puncture needle. After about 45 minutes' drainage to remove all preformed C.S.F. and when the rate of flow had become fairly constant (about one drop in from 30 to 50 sec.), the radioactive sample was injected intravenously.

In order to avoid the dilution of the newly-formed spinal fluid with the relatively large volume of pre-formed fluid, continuous open drainage from the cisterna magna was used. This technique served to eliminate the necessity of making uncertain corrections for the dilution of the newly-formed C.S.F. with the preformed, non-radioactive fluid. The animal was tilted in such a manner as to place the drainage from the cisternal puncture at the lowest possible point of the animal's central nervous system. It was felt that this facilitated as rapid a flow as possible of the C.S.F. from its point of formation to the drained cisterna magna. In this way the newly-formed C.S.F. was obtained within a minimum of time. The lowering of the spinal fluid pressure seemed insufficient to alter appreciably the composition of the fluid.

Samples of C.S.F. were collected continuously for the first two or three hours in tared tubes which were changed at timed intervals. At irregular intervals

³ The use of radioactive isotopes to label ions foreign to the body also may be advantageous because of the greater sensitivity and convenience of measurement of the radioactivity as an analytical tool over the chemical methods of analysis for many ions. The radioactive isotopes of the foreign ions, rubidium, strontium and bromine were made use of in the present investigation for this reason.

(days) the dogs were again anesthetized and cisternal puncture was performed to obtain samples of C.S.F. Samples of blood were drawn from different veins in the leg into heparin at recorded intervals after the injection and were centrifuged to obtain the plasma.

The tubes containing the samples of spinal fluid were reweighed to determine the volume obtained. The contents were then washed into a 10 ml. Coors ashing capsule and were evaporated without boiling. Aliquots of the plasma were similarly evaporated. The radioactivity of each sample was then determined by means of a Lauritsen electroscope, and after correction for decay, etc., was expressed in arbitrary units. The radioactivity per unit volume of the samples of spinal fluid was considered to represent the concentration of radioactivity at the mid-point of the interval during which the sample was collected. These concentrations, and those of the plasma similarly obtained, were plotted and smooth curves were drawn through the points. From these curves there were obtained the ratios of the concentrations of the constituents studied in spinal fluid to their concentrations in plasma.

The concentrations of the labeled ions in the blood or C.S.F. were expressed in terms of a unit which is one-millionth of the amount of the radioactivity of the isotopes administered. This is expressed mathematically by the relation:

$$\text{Units in sample} = \frac{\text{Radioactive count per ml. of sample} \times 10^6}{\text{Radioactive count in administered dose}} \quad (1)$$

The electrolyte solutions containing the radioactive isotopes were prepared according to methods that are now standard (see 9).

EXPERIMENTAL RESULTS. *Rate of formation of fluid.* The difficulties in the determination of the rate of formation of C.S.F. and the objections to the data that have been reported have been amply discussed by others (6, 7). Measurements obtained by open drainage presumably do not correspond with the normal rate because of the reduced pressure of the C.S.F. The rate of the formation of fluid even under these conditions is of interest. In our experiments some thirty dogs were studied. In these large animals the rate of flow of the C.S.F. averaged about 0.2 ml. an hour for each kilogram of body weight, or 96 ml. each 24 hours in a dog weighing 20 kgm. With a few exceptions, the range of variation was small. The maximum was about 0.3 ml. and the minimum about 0.1 ml. an hour per kgm. It is interesting that the animals in which the C.S.F. was obtained at the rate of 0.1 ml. an hour on one occasion gave an output of almost exactly the mean value in the successive experiment.

It is striking that the value of 432 ml. in 24 hours reported by Masserman (10) as the rate of formation of C.S.F. in man corresponds almost exactly to the rate of formation of C.S.F. observed in this study, when reduced to terms of milliliters an hour per kilogram of body weight.

The alkali elements. Sodium. Solutions of 1 per cent sodium chloride, in which the sodium was labeled with Na^{24} , were injected intravenously into the dogs, in amounts varying from 7 to 15 ml. and with a radioactivity value of between 100 and 400 microcuries. The curves showing the rate of disappearance

of the Na^{*4} from the blood plasma and its rate of increase in the C.S.F. are plotted in figure 1.

The doses of sodium injected produced little increase in the concentration of sodium in the blood, and consequently a large gradient of concentration was not an important influence in determining the curve of disappearance of the labeled sodium from the blood stream.

The curves of the decrease in Na^* of the blood plasma and its increase in the C.S.F. cannot be represented by simple mathematical functions. The same thing holds true for the other ions. A plot of the logarithm of the Na^* activity against time does not yield a straight line, which would be the case if the curves could be represented by simple exponential functions. An approximate mathematical analysis indicates that the curves should be represented by equations involving hyperbolic functions. The parameters of the functions are difficult to evaluate and in view of the considerable fluctuations in the experimental data, an evaluation was not attempted. In connection with the mathematical form of the curves, it is of interest that Dominguez, Goldblatt and Pomerene (11) found that the elimination of intravenously injected creatinine from the bloodstream could be represented by a hyperbolic type of equation.

The variability observed between the curves of the different experimental animals is largely to be accounted for by differences in the volume of blood and extracellular fluid. The differences in the curves are decreased but not completely eliminated if the results take into consideration body weights.

The curves of figure 1 show that in the first sampling period (from 2 to 3 min.) after intravenous administration, the average Na^* activity in the blood plasma was approximately 350 units per ml. This value dropped rapidly during the first hour and very slowly thereafter, the average at this time being about 200 units Na^* per ml. If all the Na^* were in the blood plasma, its concentration would be about 1,200 units per ml. The rapid drop in the first hour probably represents mainly an exchange of Na^* among the components that make up the extracellular fluids of the body. The slow subsequent drop represents the urinary excretion of the labeled sodium and cellular exchanges with sodium containing tissues such as bone and connective tissue.

The plasma Na^* values during the approximately stabilized period of concentration (after one hour) are suitable for calculating the mass of the body in which the sodium was distributed. Such calculations were carried out using the equation:

$$(\text{H}_2\text{O})_{\text{Na}^*} = \frac{\text{administered Na}^* \text{ minus excreted Na}^*}{\text{specific Na}^* \text{ content of plasma}} \times \frac{100}{\text{body weight}} \quad (2)$$

in which $(\text{H}_2\text{O})_{\text{Na}^*}$ represents the apparent volume of distribution of the Na^* in the body in percentage of body weight (12). A calculation of the urinary excretion during the first hours after the injection, from values for the Na^* in the urine determined at intervals of 24 and 48 hours, showed it to be a negligible factor in the calculation of $(\text{H}_2\text{O})_{\text{Na}^*}$. The calculations yielded values of between

⁴ The chemical symbol with an asterisk designates a radioactive labeled atom.

22 and 29 per cent for the apparent distribution of the Na^* with an average of about 25 per cent. The average value is approximately that found in rabbits (13) and rats (12).

The rate at which equilibrium is attained in the distribution of the Na^* in the extracellular fluid varies with the body size of the animal. In rats the concentration of Na^* in the plasma reaches a constant level in about ten minutes after administration, in dogs about one hour and in man about three hours are required (14).

Following its injection into the blood stream, the Na^* in the C.S.F. increases steadily for many hours before a maximum is reached.⁵ The concentration of Na^* in the C.S.F. eventually overtakes the concentration of Na^* in the plasma,

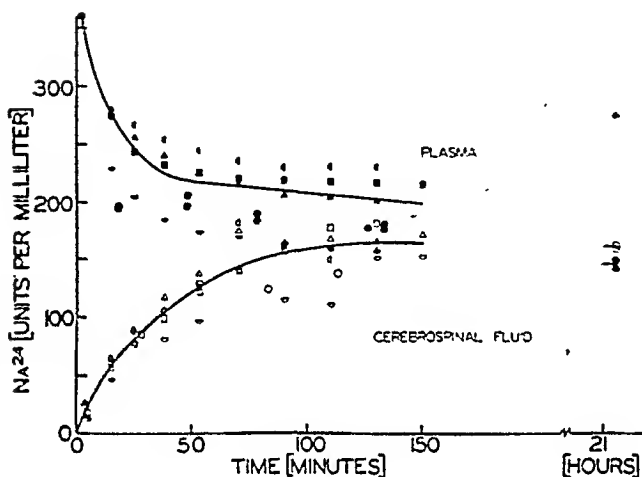


Fig. 1

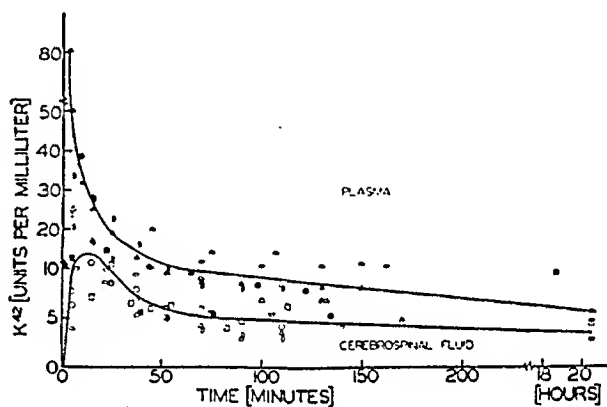


Fig. 2

Fig. 1. The rate of disappearance of labeled sodium from the blood plasma and the rate of its accumulation in freshly formed C.S.F. in normal untreated dogs. In this and in the following curves, each experiment on an animal is represented by a characteristic symbol which is solid for plasma values and open for C.S.F. The curves represent the averages of the experimental points. Unit concentration represents one-millionth of the radioactivity of the administered dose of labeled NaCl . Breaks in the abscissa represent changes in the time scale. Breaks in the ordinate represent changes in the concentration scale.

Fig. 2. The rate of disappearance of labeled potassium from the blood plasma and the rate of its accumulation in freshly formed C.S.F.

as was to be expected on the basis of their respective chemical compositions. After 21 hours the concentration of Na^* in the C.S.F. was higher than in the plasma, and the ratio of C.S.F. to plasma Na^* was 110 per cent, which comes within the range of variation of this ratio in normal human beings as determined chemically.

⁵ Staining with the dye, brilliant vital red, which reduces the permeability of the barrier between blood and C.S.F. to cocaine, does not alter the permeability of the barrier for sodium ions or water (15). The curves for the concentration of Na^* in the blood plasma and in C.S.F. of stained dogs can be essentially superimposed upon the curves of the untreated animals. The water distribution of the body is not significantly disturbed by staining with brilliant vital red. In three tests the $(\text{H}_2\text{O})_{\text{Na}^*}$ values were calculated to be 22, 25.5 and 29 per cent.

Potassium. The data of six experiments on dogs injected with K^* are plotted in figure 2. The amount injected varied between 7 and 30 ml. of an approximately isotonic solution (1.3 per cent) of KCl with a radioactivity value of between 150 and 1,000 microcuries.

The rate of disappearance of K^* from the blood plasma followed a curve that is similar in shape to that found for Na^* , but the concentrations of K^* in the plasma were always much lower. The K^* , injected as isotonic KCl, left the plasma with great rapidity. The maximum concentration obtained in the first sampling period was about 80 units per ml. of plasma. After about an hour, when the plasma K^* level tended to plateau, the average concentration was between 8 and 10 units per ml. in contrast to about 200 units found for Na^* . In other words, the highest concentration of K^* in the plasma after about an hour is only about 5 per cent of that for Na^* . At 20 hours the concentration of K^* is down to about 5.5 units per ml., the Na^* down to only 150 units. The concentration of K^* in the plasma never remained constant, but in the later periods of time the rate of its reduction became quite small.

The accumulation of K^* in the C.S.F. followed a very different curve from that found for Na^* . There was a very rapid increase in the concentration of K^* in the C.S.F. in the first ten minutes after injection. In from ten to twenty minutes the average concentration of K^* passed through a maximum and then dropped off, paralleling the course of the curve for K^* in the plasma. The first part of the C.S.F. curve indicates that the rate of permeation of potassium from blood to C.S.F. is relatively rapid. The composition of the C.S.F. probably lags behind in following the first very rapid fall in the concentration of potassium in the plasma. This may be responsible for the maximum observed in the curve of the K^* in the C.S.F.

Whereas the injection of isotonic solutions of Na^*Cl caused little increase in the concentration of sodium in the plasma, the injection of equivalent quantities of isotonic K^*Cl produces a considerable increase in the concentration of potassium on account of the normally lower level of the concentration of potassium in the plasma. This rapidly drops down to normal.

With the amounts injected in these experiments (from 7 to 30 ml.) the increase in concentration if the solutions were distributed through the blood plasma would be between 1 and 5 mM and between 0.2 and 0.8 mM if distributed throughout the extracellular fluid. The concentration of sodium in blood plasma or extracellular fluid is about 135 mM; that of potassium is about 5 mM. It is therefore evident from this that, whereas the injected material only slightly increased the concentration of sodium in the blood plasma, it produced increases of from 20 to 100 per cent in the concentration of potassium. The enhanced concentration gradient caused by the increase in the concentration of potassium in the plasma, although it is only transitory, probably has a marked effect on the initial rate of permeation of K^* into the C.S.F.

This increase in the concentration of potassium in the plasma persists for only a short period since it is very quickly taken up by the extracellular fluid and then by the cells themselves. Even when the concentration of K^* in the plasma is

80 units per ml, the volume of distribution of potassium is about 45 per cent, which is nearly double the extracellular fluid space calculated from the distribution of the stabilized Na^* concentration. When the level of K^* in the blood plasma is stabilized, the tissues have taken up a large proportion of the injected K^* .

The fraction of the injected K^* which remains in the blood and extracellular fluid becomes very small, and the concentration of K^* in the tissues attains a level many times greater than that of the plasma. This is aptly illustrated by a calculation from the results of one of several experiments in which a biopsy of muscle was obtained from the animal at various times, in this instance 30, 95 and 140 minutes after the start of the experiment. The dog weighed 30 kgm. The concentrations of K^* in the blood plasma were 15 units per ml. at 30 minutes, 9.6 units at 95 minutes and 11.6 units at 140 minutes. The concentrations of K^* in the muscle were 22 units per gram at 30 minutes, 33.5 units at 95 minutes and 42.5 units at 140 minutes. Muscle contains about 20 per cent extracellular fluid. Consequently the figures given above must be increased by about 20 per cent to obtain the intracellular concentration. Assuming the total amount of extracellular fluid, including plasma, to represent about 25 per cent of the body mass and the concentration of K^* to be the same in interstitial fluid and in the blood plasma, it may be calculated that the 7,500 ml. of extracellular fluid of the dog weighing 30 kgm. contained 7.5 per cent of the injected K^* . The remaining 92.5 per cent must have had an intracellular distribution. If the distribution were uniform throughout the rest of the body, approximately 40 units of K^* per gram of cellular body tissue would be found. Furthermore, since bone contains but little potassium, a higher concentration of K^* would be expected in the soft tissues than is obtained on the assumption of an equal concentration after subtracting the mass of the body in the form of extracellular fluid. The calculation given above supports the deduction that a small fraction of the potassium administered is retained in the extracellular constituents and that the major portion is exchanged with the potassium of the cellular constituents.

Rubidium. A study of rubidium from the standpoint of its ability to penetrate the blood-C.S.F. barrier is of interest because, of all the alkali elements, it most closely parallels potassium in its physiological behavior. Inasmuch as the half life of radioactive rubidium (18 days) is considerably longer than that of K^{42} , information may be obtained at a later date with radioactive rubidium than is possible with radioactive potassium. Such information should be of value in estimating the physiological behavior of potassium because of the parallelism in the physiological behavior of the two ions.

The radioactive rubidium employed was Rb^{86} , 88 obtained by bombardment of rubidium chloride with neutrons. After a preliminary trial, two successful experiments were carried out on two dogs, one weighing 26, the other 28 kgm. Fifteen milliliters of isotonic Rb^*Cl (2 per cent) was administered to the first and 10 ml. to the second animal. In each instance the radioactive value was about 300 microcuries. The result of these two experiments are plotted in figure 3.

The curves for the elimination of Rb^* from the blood plasma and for its ac-

cumulation in the C.S.F. closely resemble those of potassium. There was a similar rapid initial drop followed by a slow but continuous reduction in the concentration of Rb^* in the plasma. As in the case of K^* ; the rate of lowering of the Rb^* concentration in the plasma becomes quite small after the rapid initial drop. The larger part of the rubidium was soon removed from the extracellular fluid and, like potassium, it must be taken up by the soft tissues of the body, probably by exchange with potassium.

Calculation of the volume of distribution of the Rb^* showed that it quickly surpassed the volume of extracellular fluid in the body and that the apparent volume increased throughout almost the entire duration of the experiment. From this, one may conclude that the rate of penetration into the cells is a relatively slow process and that a steady state is not quickly reached.

The curve of the accumulation of Rb^* in C.S.F. is much like that for K^* . The concentration of Rb^* in the C.S.F. reached a maximum in about half an

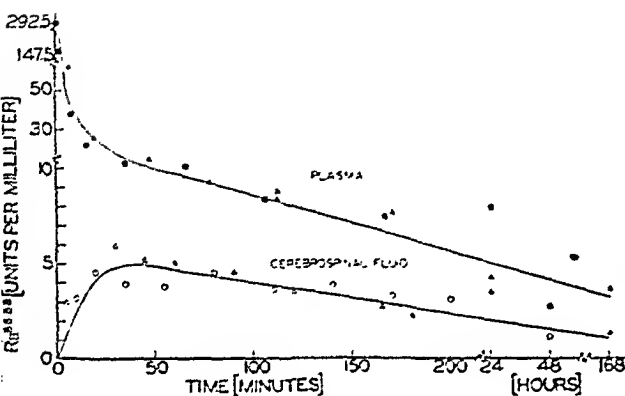


Fig. 3

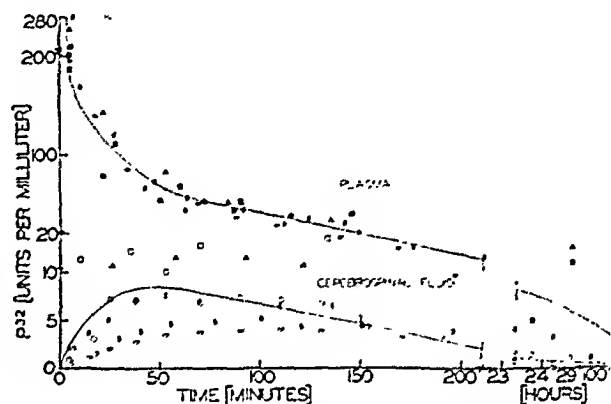


Fig. 4

Fig. 3. The rate of disappearance of labeled rubidium from the blood plasma and the rate of its accumulation in freshly formed C.S.F.

Fig. 4. The rate of disappearance of labeled phosphate from the blood plasma and the rate of its accumulation in freshly formed C.S.F.

hour and then decreased gradually. The maximum level of concentration attained by Rb^* , however, was only about half as high as that found for K^* .

The behavior of Rb^* was similar to that of K^* in one other respect, namely, that the rate of urinary excretion of both these elements in the amounts administered was nearly linear and amounted to 1 to 2 per cent of the administered dose per day.

The experiments show that Rb^* does not penetrate the barrier between the blood and C.S.F. as readily as does K^* . The concentration of Rb^* in the C.S.F. was at all times lower than the corresponding concentration of K^* in the C.S.F., and the ratios between the Rb^* in the plasma and that in the C.S.F. were somewhat less than those of K^* . This may be due to the larger ionic diameter of the rubidium ion. On the other hand, it may be the result of differences in the properties of potassium and rubidium salts of physiologically important compounds, e.g., lipids. Such an explanation has been offered for the difference in

permeability between sodium and potassium. In the present state of knowledge, no acceptable explanation can be offered for the physiological differences between ions of the alkali elements.

Plurivalent ions. The valency of an ion is one of the factors which affects its permeability. Plurivalent ions of biological importance are the phosphates and the ions of the alkali earth elements. In the course of this investigation experiments were carried out with phosphate and strontium ions.

Phosphate. The data obtained by the use of phosphate are plotted in figure 4. Six experiments were carried out with phosphate labeled with P^{32} . The usual dose of phosphate injected contained 40 mgm. of P^* in a volume of 10 ml. with a radioactive value of from 200 to 500 microcuries. While this dose is small, it is sufficient to double the concentration of P in the blood plasma. The first sampling periods showed a comparatively high content of P^* in the blood plasma (280 units per ml.). This indicates that the phosphate is taken out of the plasma more slowly than is potassium. The concentration of labeled phosphate in the plasma drops off continuously, very steeply in the first hour and then more slowly. There is almost no tendency for it to reach a plateau level such as is found with the alkali elements.

Two factors which do not operate in the case of potassium may be offered in explanation of this. One is that while phosphate is chiefly an intracellular constituent like potassium, it differs from potassium in not continuing to exist as the inorganic ion but instead is mainly converted into phosphate esters of various organic compounds. By this process the concentration of phosphate ion is maintained at a low level and the tendency of the phosphate to leave the plasma and enter the tissues is maintained longer. The second factor is that bone finally becomes the chief site for the storage of administered phosphate and the skeleton forms a great reservoir for the accumulation and exchange between the labeled and unlabeled forms of phosphate. As is the case with potassium, the bulk of the administered P^* is soon removed from the blood plasma and extracellular fluid but the rate of removal is considerably slower than that found for K^* .

The curve for the appearance of labeled phosphate in the C.S.F. shows a slower rate of increase than was found in the case of potassium, but, like the potassium, the concentration of labeled phosphate passes through a maximum. The average time at which this appears is much later for phosphate. In the case of P^* the maximum usually appeared in about one hour; in some experiments it took from 2 to 2.5 hours to reach the maximum.

After 24 hours, the concentration of P^* in the C.S.F. dropped to less than one unit per ml. These low values were, of course, difficult to estimate accurately, and the readings of these periods are subject to great errors.

There is also a great individual variation to the permeation of phosphate. Figure 4 shows that, while the points fall very nearly on the same curve for the decrease of P^* in the plasma in the first four hours after injection of equivalent doses into the blood stream, the concentration of P^* in the C.S.F. may vary from 4 to 16 units per ml. at the time when the P^* in the C.S.F. reaches the peak of its concentration.

Strontium. Information on the permeability of the blood-C.S.F. barrier to the alkali earth cations which are constituents of the C.S.F., namely, calcium and magnesium, would be very valuable in a comprehensive study of the properties of this barrier. Unfortunately, no radioactive isotopes of these elements suitable for such an investigation are now available. There is available, however, a very strongly radioactive isotope of strontium (Sr^{89}) with a long half life (55 days) which is ideal for tracer studies. Since the accumulated information indicates that the physiological behavior of strontium in tracer doses parallels that of calcium (16), experiments were undertaken with strontium ions in order to secure information from which deductions could be made as to the probable ability of calcium to permeate the barrier.

Sr^{89} was obtained by bombardment of metallic strontium and was administered as a solution of strontium lactate. The dose varied between 250 and 500 mgm. of the salt per animal. The curves of the results of three experiments are plotted in figure 5. The curve for the disappearance of strontium from the blood plasma shows that the concentration tended to reach a constant level at about 100 minutes after the injection. Subsequently the concentration of Sr^* in the plasma dropped off slowly, as shown by specimens taken at intervals of several days. After nine days, the concentration was reduced to between 2 and 3 units per ml. of plasma.

This continuous reduction in the concentration of the Sr^* in the plasma is partly to be accounted for by the removal of strontium from the extracellular fluid by the skeleton and partly by excretion. Specimens of urine and feces were collected for periods of nine days following the administration of the strontium salt. The amount excreted in the urine was considerable in the first four days (an average of about 20 per cent), but very little appeared in the urine thereafter. Fecal excretion occurred at a slower but more continuous and nearly linear rate; at four days the average fecal excretion was about 10 per cent and at nine days 22 per cent of the administered dose. At four days the total excretion amounted to about 30 per cent and at nine days, 40 per cent of the administered dose of Sr^* .

If strontium were uniformly distributed throughout the body, the decrease in the concentration of the plasma Sr^* should be proportional to the amount excreted. Actually, a much greater reduction occurred in the concentration of plasma than can be accounted for by the excretion. After nine days, the Sr^* in the plasma was reduced to about 2 per cent of the concentration at 100 minutes. If excretion was the only factor, the concentration would hardly have been halved. Consequently, there must be a slow removal of Sr^* from the circulating body fluid other than that occurring by excretion. This is explained by the fact, as already mentioned, that Sr^* is in large part removed from the circulation by the skeleton. In adult animals such as were used in these experiments, the uptake of strontium by the bone is probably brought about by an exchange with calcium ions.

Calculation of the volume of distribution of Sr^* in the body, assuming it is uniformly distributed at a concentration equal to that in the blood plasma, yielded values of between 35 and 50 per cent of the body mass during the plateau

period. This indicates that at first there is a rapid distribution of the injected Sr^* throughout the extracellular fluids of the body. This is followed by a slower removal of the Sr^* by the body tissues and in particular the skeletal system.

The curve for the accumulation of Sr^* in the C.S.F. shows that the maximum of concentration was reached in about one hour. Following this, there was a very slow but continuous drop in concentration which paralleled the reduction that occurred in the plasma Sr^* . The concentration of Sr^* in the C.S.F. always remained below that of the plasma.

The results obtained with strontium give a clue to the expected behavior of calcium with respect to the C.S.F. While a radioactive calcium (Ca^{45}) of long half life (180 days) is available, the extreme softness of its radiations and the minute amount taken up by the brain makes it unsuitable for study of the permeability of the blood-C.S.F. barrier. The physiological behavior of strontium resembles that of calcium. Reasoning by analogy, it appears likely that the

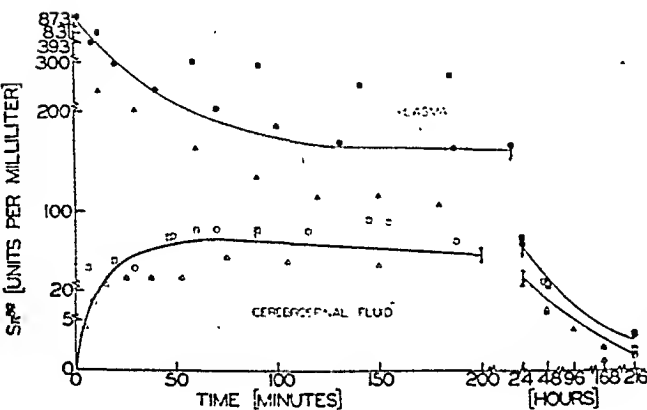


Fig. 5

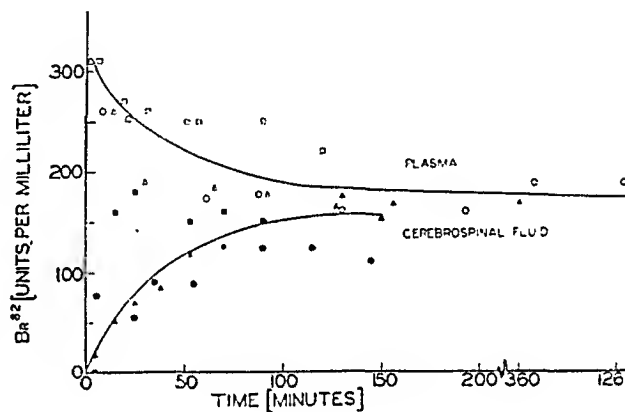


Fig. 6

Fig. 5. The rate of disappearance of labeled strontium from the blood plasma and the rate of its accumulation in freshly formed C.S.F.

Fig. 6. The rate of disappearance of labeled bromine from the blood plasma and the rate of its accumulation in freshly formed C.S.F.

curves for the elimination of calcium from the blood plasma and its accumulation in the C.S.F. would closely resemble those obtained with Sr^* . The rate of disappearance of calcium from the blood stream would probably be somewhat more rapid because calcium is taken up more completely by the skeleton (16) than is strontium. The rate of accumulation of calcium in the C.S.F. would also perhaps be somewhat faster.

The halides. Chloride is one of the major ionic constituents of the C.S.F. Unfortunately, the short half life of the best-known radioactive isotope of chlorine (Cl^{38} , half life = 37 minutes), makes the use of this ion impractical in tracer studies on the permeability of the barrier between the blood and the C.S.F. On the other hand, radioactive isotopes of bromine (Br^{82} , half life = 34 hours) and iodine (I^{131} , half life = 8 days), are available, which are quite suitable for tracer studies. The permeability of the blood-C.S.F. barrier to bromide and iodide accordingly was investigated with these labeled ions.

Bromide has never been established as being an essential constituent of the body (17), and the amount of iodide that is present in the circulation is extremely minute. The permeability of bromide and, to a lesser extent, of iodide has been extensively investigated by ordinary chemical methods. This has necessitated the administration of fairly large doses of the salts of these ions and in amounts far in excess of those normally present.

It is readily possible to obtain radioactive bromine and iodine of extremely high specific radioactivity, which makes it feasible to study the partition of these ions in concentrations that are within the range of those usually found in the body and in concentrations very greatly below those it is possible to estimate by ordinary chemical methods. The present study was undertaken in order to determine whether the use of minute doses of the halides would alter in any particular the findings that have been obtained with massive doses.

The partition of bromide and iodide in the body by means of chemical methods of analysis has been the subject of recent investigation by a number of authors (18-20). Except in the central nervous system, these halides were found to pass rapidly into the extracellular fluid of the body and to parallel chloride in their distribution and partition. The rate of passage of bromide and iodide from the circulating blood plasma into the C.S.F. was found to be slow and the concentration of these ions in the C.S.F. never reached the heights found in the blood plasma. Mason (18) observed that the concentrations of bromide in the C.S.F. were considerably lower than in the plasma many days after the last ingestion of bromide in human subjects suffering from bromide intoxication. Following the intravenous administration of a single dose of bromide, Wallace and Brodie (20) found that the ratio of bromide in the C.S.F. to that in the plasma tended to persist at the value of 83 per cent for from 24 hours to 13 days. The rate of passage of iodide into the C.S.F. and the accumulation in the C.S.F. were observed to be considerably below corresponding values for bromide. No evidence of a constant ratio of concentration between C.S.F. and plasma was found by Wallace and Brodie for iodide. These authors obtained evidence that a certain minimum concentration of iodide must be attained in the plasma before it passes into the C.S.F.

Bromide. For the present experiments the radioactive isotope Br^{82} was prepared by bombarding bromobenzene containing a small portion of aniline according to the method of Lu and Sugden (21). The Br^{82} which was formed was extracted with water, converted to bromide and finally prepared for administration in an isotonic sodium chloride solution. Consequently, the bromide administered consisted of the Br^{82} liberated by the bombardment and the bromide impurity in the sodium chloride.

The results of three experiments carried out with Br^* are plotted in figure 6. The plasma and C.S.F. curves of bromide closely resemble the ones found for sodium. The blood plasma Br^* concentration in the first sample was about 300 units per ml. The plasma concentration then dropped off until it reached the value of about 175 units in about one and one-half hours. This level persisted with little further reduction for a very long time. The prolonged plateau level

of Br^* concentration found in the blood plasma is evidence that a complete distribution of bromide in the body is reached within a period of one or two hours. The short time required to reach a steady state and the prolonged period of constant concentration in the plasma is evidence for the view that the distribution of bromide, like that of chloride, is essentially extracellular. Calculation of the distribution of the Br^* in the body yielded values of between 22 and 25 per cent of the mass of the body. The elimination of Br^* from the blood plasma and its distribution in the body closely parallels that found for sodium with Na^{24} .

The concentration of Br^* in the C.S.F. increased as the plasma concentration dropped and it gradually approached but never equalled the plasma concentration. A plateau level of Br^* concentration in the C.S.F. was attained in about two hours.

Iodide. The isotope, I^{131} , used in the iodine experiments was prepared by bombardment of tellurium as described by Hamilton and Soley (22). The iodine prepared in this manner contains a high concentration of the radioactive isotope, which makes it possible in spite of extremely minute doses to follow accurately the iodine and to determine its fate by the radioactive technique.

The curves for the disappearance of I^* from the blood plasma and its appearance in the C.S.F. are plotted in figure 7. The figure shows that the curve for the elimination of iodide from the blood plasma is very different from that found for bromide. There was a continuous decrease in the concentration of plasma I^* with no definite evidence of its attaining a plateau level. In this respect the iodide paralleled the behavior of phosphate. It seems probable that the I^* progressively disappears from the circulation because in the doses used it is being continuously taken up by the thyroid gland, as has been shown by, among others, Hamilton and Soley (22).

The rate of increase in the concentration of the I^* in C.S.F. was found to be highly variable. On the average, as is shown in figure 7, the concentration in the C.S.F. reached a constant level in from one to two hours. The accumulation of iodide by C.S.F. was much below that of bromide.

C.S.F. to plasma ratios. The curves of the ratios of the concentrations of the labeled ions in the C.S.F. to the concentrations in the plasma are plotted in figure 8. The curves represent the averaged values of the ratios for each individual experiment. The values are expressed in per cent. Figure 8 shows that the rapidity of the initial rate of increase in the ratios follows the order, potassium > sodium > bromide > rubidium > strontium > phosphate > iodide.

Values equal to the normal range of the C.S.F. to plasma ratios found by chemical analysis are reached only very slowly. In the case of the alkali elements, the average of the chemically determined concentration ratios are 105 for sodium and 65 for potassium. Curve 1 in figure 8 shows that the ratio for Na^* is about 60 per cent at one hour and 80 per cent at two hours. Only after 20 hours is the value of 110 reached, which is in the range of variation of the chemically determined ratio.

The ratio of K^* increases most rapidly of all at first, attaining a value of 50 per cent in 30 minutes (curve 2). From then on it increases very slowly and a

period of 18 hours is required for it to reach 62 per cent, which is in accord with the ratio found by chemical analysis. The Rb^* ratios parallel, but always remain somewhat lower than those of K^* . This indicates, as has been found by other physiological tests, that although rubidium shows certain similarities to the potassium in biological properties, differences do exist and physiologically potassium is not altogether replaceable by rubidium (23).

The ratio of P^* attains a value of 15 per cent in the first hour, 23 per cent in two and one-half hours, and then apparently tends to fall off. The decrease may be fictitious because of the very low concentrations of P^* remaining in the plasma in the later time intervals. Because of the low radioactive intensity, the probable errors of the measurements are greatly enhanced.

The ratio of C.S.F. to plasma Sr^* increased most rapidly during the first hour. While the concentration of Sr^* in the C.S.F. reached a maximum in about one

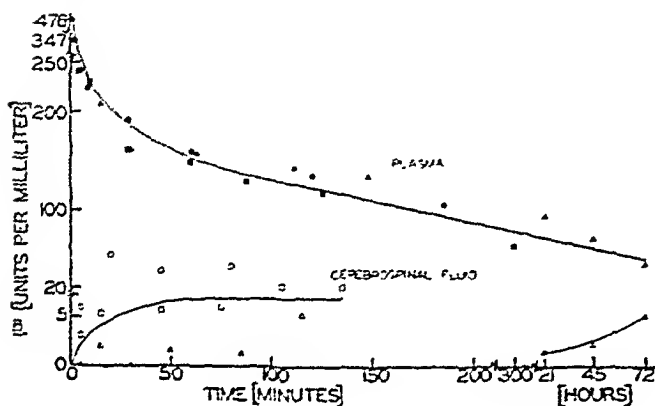


Fig. 7

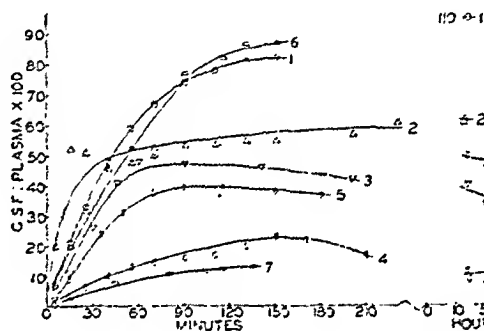


Fig. 8

Fig. 7. The rate of disappearance of labeled iodine from the blood plasma and the rate of its accumulation in freshly formed C.S.F.

Fig. 8. Comparison of the curves showing changes in the average C.S.F. to plasma ratios with time. The numbers of the curves represent the following labeled ions. 1, sodium; 2, potassium; 3, rubidium; 4, phosphate; 5, strontium; 6, bromide; 7, iodide.

hour, the ratio of C.S.F. to plasma Sr^* did not reach a maximum until after 24 to 48 hours. At the end of an hour the ratio was about 40 per cent; from 24 hours on it remained about 50 per cent. The value of 50 per cent is of interest because this is the ratio of C.S.F. to serum calcium normally found by chemical analysis. In this respect strontium closely mirrors calcium.

Curve 6 of figure 8 shows that the ratios of Br^* increase at about the same rate as the Na^* ratios. The values of the ratios approach but do not reach a constant value in two hours. At two hours, the average ratio was 85 per cent, at two and one-half hours it was 87 per cent. These values are only a little higher than the ratios found by Wallace and Brodie (20) by chemical analysis. It may be concluded from these results that the rate of penetration and partition of bromide between blood and C.S.F. is essentially the same for minute as for large doses.

Of the ions studied, iodide was the least permeable to the blood-C.S.F. barrier. The maximum ratio of concentration of I^* between C.S.F. and plasma was only

12 per cent in these experiments (curve 7). This is less than one half of the maximum ratio observed by Brodie and Wallace upon the administration of 100 m-eq. of NaI to a 15-kgm. dog. The partition of iodide between blood and C.S.F. is evidently affected to a considerable degree by the size of the administered dose. An explanation for this very low accumulation of I^* in the C.S.F. may be that while in the C.S.F. the iodine is present in a completely diffusible form, in the blood plasma a considerable proportion of the iodine is united to protein and is non-diffusible. Because of the minute amount administered in the present experiment, a large proportion is converted into the non-diffusible iodide. This would serve to explain the difference between the results obtained in these experiments and those of Brodie and Wallace upon the administration of the massive dose of 100 m-eq. of sodium iodide.

The present experiments demonstrate that the difficulty of permeation of the halides into the central nervous system increases in the order of bromide to iodide. This result is consistent with other studies that give the order of permeability as chloride > bromide > iodide. Although this corresponds to their order of ionic diameter, it seems quite likely that other factors, the nature of which is at present unknown, are also of great importance in determining the specific permeability of the halides into the central nervous system.

DISCUSSION. The most important aspect of the present study is the bearing it may have on the question of whether the C.S.F. is formed by passive diffusion or by active secretion. Extracellular fluid as exemplified by lymphatic or capillary transudates is probably formed by diffusion or simple ultrafiltration. The composition of the cellular elements, on the other hand, is probably maintained by secretory mechanisms.

A comparatively rapid exchange of diffusible constituents and the attainment of a steady state which closely approximates a true thermodynamic equilibrium characterize biological diffusion as judged from experiments on extracellular fluid. Ion specificity apparently is an unimportant factor.

On this basis the formation of C.S.F. must be a matter of secretion rather than of passive diffusion. The experiments reported here show that the rate of accumulation in the C.S.F. of the injected labeled ions is selective and is a comparatively slow process. The rate of accumulation in the extracellular fluid is at least ten times more rapid. When a steady state with respect to concentration is reached, the ratios of the labeled ions between C.S.F. and plasma indicate that a thermodynamic equilibrium has not been attained.

Following the lapse of a considerable length of time the ratios of the labeled ions between C.S.F. and plasma reach values equivalent to those obtained by chemical analysis. Most of the chemical analyses have been carried out on spinal fluid obtained by lumbar puncture. It may be objected that this differs in composition from freshly formed C.S.F., due to exchange of solutes in the course of the sluggish circulation of the C.S.F. The C.S.F. obtained in this work represents material that was as freshly formed as it was possible to obtain. The cisternal puncture and precaution of draining off the C.S.F. before starting the

test greatly reduced the reabsorption of the newly-formed C.S.F. and its constituents.

It seems reasonable, then, that the chemical composition of normal C.S.F. is representative of a steady state. This steady state, however, obviously is not a thermodynamic equilibrium. The attempts to explain away the unusual composition of the C.S.F. by assuming unique effects on the activities of the constituent ions are not convincing, because, if such assumptions were true, these unique effects on the activities should be manifest in capillary ultrafiltrates.

The endothelium elements in the capillaries apparently act approximately as membranes impermeable only to certain colloids (mainly protein). Cellular structures more complex than this, such as are presumably concerned with the elaboration of the C.S.F., have an active secretory function. According to the classical theory of the formation of C.S.F., the choroid plexuses are the chief secreting elements; according to that offered by Wallace and Brodie the secretion takes place at continuations of the glial membranes of the pia, which surround the precapillary blood vessels of the brain.

The view that the more complex the cellular structure, the slower is the exchange of dissolved materials, is supported by the recent observations of Flexner and Pohl (24) on the transfer of radioactive sodium across the placenta.

Acknowledgment. We are greatly indebted to Prof. E. O. Lawrence and the staff of the Radiation Laboratory of the University of California for supplying us with the radioactive elements used in this investigation.

SUMMARY

1. The permeability of the barrier between blood and cerebrospinal fluid to ions has been studied by means of tracer experiments with their induced radioactive isotopes. The investigated ions were sodium, potassium, phosphate and iodide which are normally present in the body and rubidium, strontium, and bromide which are foreign to the body. Determinations were made of the rates of disappearance of the intravenously injected ions from the circulating blood plasma, and the rates of their accumulation in freshly formed C.S.F.

2. Similar curves were obtained for the disappearance of all the injected ions from the blood plasma. The rapidity of the disappearance of the ions from the blood plasma decreased in the order potassium = rubidium > phosphate > iodide > sodium = bromide > strontium. The levels of labeled sodium, bromide and strontium tended to plateau after about one hour. Calculations from the plateau concentrations gave results which indicated that the injected labeled sodium and bromide became exclusively and uniformly distributed throughout the extracellular fluids of the body. The curve of the disappearance of strontium from the blood plasma indicated that it was first rapidly distributed in the extracellular fluids of the body and then slowly taken up by the body tissues, particularly the skeletal system. Potassium and rubidium showed less of a tendency, and phosphate and iodide no tendency to reach a plateau concentration. After about one hour, only about 5 per cent of the injected

labeled-potassium and rubidium was present in extracellular fluids and the remainder, less the amount excreted, accumulated in the intracellular phase of the soft tissues of the body at a concentration about ten times greater than in the plasma. The bulk of the labeled-phosphate was taken up by the mineral matter of the bone and by the intracellular phase of the soft tissues where it was converted into various organic esters. The labeled-iodide was probably mainly taken out from the blood stream by the thyroid tissue.

3. The rate of increase in concentration of the labeled-ions in the C.S.F. followed the order potassium > sodium > bromide > rubidium > strontium > phosphate > iodide. The C.S.F. concentration curves of potassium, rubidium, and phosphate exhibited pronounced maxima at about 20, 30 and 60 minutes respectively. Strontium exhibited a less pronounced maximum and sodium no maximum in the C.S.F. concentration curve.

4. The ratios of the C.S.F. to plasma concentrations of the labeled ions slowly approached the values found by chemical analysis. Many hours were required to reach values equivalent to the chemically determined ratios. The delay was selective, and varied greatly for the different ions.

5. The C.S.F. to plasma concentration ratios were always lower for rubidium than for potassium, indicating that the blood-C.S.F. barrier is less permeable to rubidium than to potassium. From the close physiological relationship of strontium to calcium it was deduced that calcium would have about the same degree of permeability to the blood-C.S.F. barrier as strontium. The permeability of the barrier to the halides probably decreases in the order chloride, bromide and iodide.

6. The hindrance to the free passage of ions from the blood stream to the extracellular fluid of the central nervous system and the deviation from the Donnan law of distribution of the steady ratios of the concentrations of these ions between the C.S.F. and the plasma constitute evidence that the exchange between the blood and brain takes place by a process of secretion and not by simple diffusion or ultrafiltration.

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EFFICACY OF ISOTONIC SODIUM CHLORIDE AND GLUCOSE SOLUTIONS IN PREVENTING SHOCK FOLLOWING VENOUS OCCLUSION OF A LIMB IN THE DOG¹

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The use of crystalloid solutions as blood volume augmentors in the treatment of shock has been extensively reviewed (1). Generally, they have been found to be less effective than whole blood, plasma, or serum when used in the later stages of shock after capillary permeability has been altered (2). Under such conditions, the crystalloids rapidly escape from the blood stream carrying plasma proteins with them (2, 3, 4), thus actually being harmful by lessening the effect of the plasma proteins on the osmotic pressure of the blood, and possibly also by decreasing the quantity of these proteins available for body tissue needs. Nevertheless, it has been claimed that crystalloid solutions are beneficial, if administered before capillary permeability has been changed, in combating dehydration so as to *prevent* shock (2, 5). The two crystalloids most commonly employed are sodium chloride and glucose, either alone or in combination.

The venous occlusion method for the production of shock (6, 7, 8, 9) readily lends itself for the testing of the efficacy of the timely administration of glucose and saline solutions, since the course in untreated animals is consistent and uniform. As has previously been reported untreated dogs become listless, develop hemoconcentration, a fall in blood pressure, tachycardia, tachypnea and die in from 3 to 21 hours (6, 7). Any form of therapy preventing this chain of events can be considered to be effective. Furthermore, this method is particularly favorable to test the early use of such crystalloid solutions since it is evident that the development, progression and final irreversibility of shock thus produced is dependent upon the early loss of protein-free fluids from the blood, even though proteins and whole blood subsequently escape into the occluded limb. If this escape of fluid is decreased by the application of a rigid cast to the occluded limb, shock does not develop (8). It therefore appears that the fatal course could be avoided if the fluid loss could be replaced and the circulating blood volume maintained. In this report we wish to present our experience with the timely use of isotonic solutions of saline and of glucose.

PROCEDURE. In this study the operative procedure employed to produce shock was identical to that previously reported (6, 7, 8, 9). Under ether anesthesia the common and internal iliac veins of one hind-limb were ligated aseptically, and 10–15 cc. of a 1:10 suspension of lampblack³ in saline was injected

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³ The particles were 55 to 60 μ in size.

under pressure into the veins below the ligation. For later injection of fluids, a cannula closed with a trocar was tied into the brachial vein of one forelimb. The animal was then permitted to come out of the anesthesia, and observations were made of its condition as in the preceding studies (6, 7, 8, 9); these included changes in heart rate, blood pressure, hematocrit, and in the circumference of the occluded limb. In the animals not surviving, and in those sacrificed at a later time, the weight of the edematous limb was compared with that of the contralateral one in the manner previously described (6).

The isotonic solutions employed were NaCl in triple distilled water and glucose in distilled water.⁴ These solutions were injected at various postoperative intervals. The saline solution was injected intravenously in amounts of from 300 to 2500 cc. (cf. table 1). The injections were begun $\frac{1}{2}$ to 2 hours after operation and repeated every 1 to 3 hours except for the last injection which, on several occasions, was given after a lapse of as long as 7 hours, and in 3 instances was subcutaneous. The amount of each injection varied from 100 to 300 cc. (except in dog 1 in which 600 cc. injections were given twice). The quantities of glucose given were of the same order as of saline although the variation in amount administered was less. The actual quantity varied from 400 to 800 cc. given intravenously, starting $\frac{1}{2}$ hour after operation and repeated every 1 to 3 hours, with the last dose delayed sometimes for as long as 6 hours (cf. table 2). The amount of each injection ranged from 150 to 250 cc.

RESULTS. The pertinent data are summarized in tables 1 and 2 for the isotonic saline and the isotonic glucose experiments respectively.

It will be seen from table 1 that saline not only prevented the occurrence of death in all 12 dogs in which it was used, but also prevented the marked blood pressure decrease and hemoconcentration observed in the untreated animals (6, 7). Its effect occurred despite a 20 to 60 per cent enlargement of the circumference of the occluded limb in the first 12 hours, and despite an increase in leg weight (2.8 to 4.7 per cent of body weight) in the four animals sacrificed on the third to sixth post-operative day. These increases in leg circumference and weight were in the range seen in untreated dogs (6, 7), indicating that the local loss of fluid was not increased by administration of saline solution.

It will be seen from table 2 that isotonic glucose in distilled water was definitely beneficial. The mortality in the first 20 hours was 42 per cent (5 out of 12) in the animals receiving glucose, as compared with a mortality of 90 per cent in untreated dogs to date (19 out of 21). However, the results with glucose were not as satisfactory as with isotonic saline, in which the mortality in the first 24 hours was 0 per cent. The increase in leg circumference in the first 12 hours was of the same order in the glucose treated dogs (11 per cent to 68 per cent) as in the dogs receiving isotonic saline. The increase in leg weight of the dogs receiving glucose which survived and were later sacrificed was also of the same order (1.7 per cent to 6.3 per cent of body weight) as in the dogs receiving isotonic saline. As expected, the animals which died within 20 hours showed a greater increase in leg weight (3.3 per cent to 8.2 per cent of body weight), but this was

⁴ Generously furnished by the Abbott Laboratories.

TABLE 1

Effect of isotonic saline solution on the course of events following venous occlusion of the hind-limb of the dog

DOG NO.	WEIGHT	ISOTONIC SALINE INJECTED INTRAVENOUSLY			FATE OF ANIMAL IN 1ST 36 HOURS POST-OPERATIVELY	INCREASE IN WEIGHT OF LEG AS PER CENT OF BODY WEIGHT	MAXIMUM INCREASE OF THIGH CIRCUMFERENCE OVER CONTROL VALUE WITHIN 1ST 12 HOURS	CHANGES IN HEMATOCRIT POST-OPERATIVELY	CHANGES IN BLOOD PRESSURE POST-OPERATIVELY
		Time post-operatively	Quantity injected	Total injection					
	kgm.	hrs.	cc.	cc.			per cent		
1	19.9	1	150	2500	Survived		48	Increase	Slight drop
		2½	250						
		3½	600						
		5½	600						
		7	300						
		9	300						
		12	300						
2	10.9	2	300	1200 (150)*	Survived		43	Slight increase	No change
		3	300						
		5	300						
		7	150						
		10	150*						
3	11	1	100	900 (200)*	Survived		20	Temporary slight increase	Slight drop
		2	200						
		4	200						
		6	200						
		12	200*						
4	5.6	1	150	300	Survived		60	No change	No change
		2	150						
5	9.1	1	150	350	Survived		61	No change	Slight rise
		4	100						
		7	100						
6	11.4	½	200	600 (100)*	Survived		56	Slight increase	Rise
		3	150						
		5	150						
		12	100*						
7	10	½	200	1065	Survived	3.9 (on 6th day post-operatively)	32	No change	Slight rise
		3½	365						
		5½	500						
8	11.8	½	200	800	Survived	2.8 (on 3d day post-operatively)	27	Slight decrease	Drop
		3½	300						
		5½	300						
9	11.7	½	250	900	Survived	4.7 (on 3d day post-operatively)	20	No change	Slight rise
		3	300						
		4½	200						
		7½	150						
10	7.2	½	250	700	Survived		31	No change	No change
		3	250						
		8	200						
11	7.9	½	250	700	Survived		44	Slight increase	Slight rise
		3	250						
		5	200						
12	8.1	½	250	700	Survived	3.9 (on 3d day post-operatively)	47	Slight decrease	No change
		3½	200						
		5½	150						
		7½	100						

* Subcutaneously.

TABLE 2

Effect of isotonic dextrose solution (made up in distilled water) on the course of events following venous occlusion of the hind-limb of the dog

DOG NO.	WEIGHT	ISOTONIC DEXTROSE INJECTED INTRA-VENOUSLY			FATE OF ANIMAL IN 1ST 36 HOURS POST-OPERATIVELY	INCREASE IN WEIGHT OF LEG AS PER CENT OF BODY WEIGHT		MAXIMUM INCREASE OF THIGH CIRCUMFERENCE OVER CONTROL VALUE WITHIN 1ST 12 HOURS	CHANGES IN HEMATO-CRIT POST-OPERATIVELY	CHANGES IN BLOOD PRESSURE POST-OPERATIVELY
		Time post-operatively	Quantity injected	Total injection		In animals that died	In animals that survived			
	kgm.	hrs.	cc.	cc.				per cent		
1	10	$\frac{1}{2}$ 3 5 11	200 200 200 200	800	Survived		6.3 (on 3d post-operative day)	40	Increase	Drop
2	7	$\frac{1}{2}$ 4 6 10	150 150 150 200	650	Died in 13 hours (in shock?)	8.0		27	Slight increase	Rise then drop
3	8.6	$\frac{1}{2}$ 4 7 8 14	150 150 150 150 150	750	Survived		2.9 (on 4th post-operative day)	38	Increase	No change
4	10	$\frac{1}{2}$ 5	200 200	400	Survived		1.7 (on 9th post-operative day)	20	Slight increase	Slight rise
5	11.4	$\frac{1}{2}$ 2 4 $\frac{1}{2}$	200 150 150	500	Died in 10 hours in shock	3.6		30	Increase	Drop
6	12.3	$\frac{1}{2}$ 2 4 $\frac{1}{2}$ 9 $\frac{1}{2}$	200 150 150 150	650	Survived		3.9 (on 11th post-operative day)	23	Increase	Slight drop
7	10.5	$\frac{1}{2}$ 3 7	200 150 150	500	Survived			68	Increase	Drop
8	7.2	$\frac{1}{2}$ 3 6 7	250 250 150 150	800	Died within 20 hours in shock	8.2		25	Increase	Drop
9	7.7	$\frac{1}{2}$ 3 4 $\frac{1}{2}$	250 250 200	700	Survived		3.9 (on 3d post-operative day)	11	Slight increase	Slight drop
10	5.9	$\frac{1}{2}$ 2 5	250 250 200	700	Survived			61	Slight increase	No change
11	9	$\frac{1}{2}$ 2 4	250 250 250	750	Died within 20 hours in shock	6.3		21	No change	Drop
12	7.5	$\frac{1}{2}$ 2 5 $\frac{1}{2}$	250 250 150	650	Died within 20 hours in shock	3.3		11	Slight decrease	Drop

in the same range as in the untreated dogs which died in this time period (3.5 per cent to 6.9 per cent of body weight (6, 7)). The 4 dogs succumbing in shock died at the longer time interval found in untreated animals, thus suggesting that while the glucose solution did not prevent the postoperative course of events in these dogs, it probably retarded it.

In addition to the occurrence of early fatalities, the dogs receiving glucose solution had a greater trend towards hemoconcentration and hypotension than did the dogs receiving saline solution, suggesting that even in those animals which survived, the glucose solution was not as effective as saline in preventing the early stages of shock. The difference in effect between saline and glucose does not appear to depend upon the manner or quantity of fluid administration.

DISCUSSION. Several facts stand out from this study.

1. Replacement of fluid loss by isotonic crystalloid solutions is effective in this type of shock when begun before capillary permeability is definitely altered. The results support the deductions previously arrived at (6, 7, 8, 9) that the venous occlusion method of producing shock initiates the fatal chain of events by loss of fluids from the blood into the occluded limb. Our experience shows that isotonic solutions of crystalloids have no demonstrable effect on the escape of fluid from the blood when administered early, if the failure to obtain a more rapid enlargement of the limb with occluded veins is a good criterion.

2. Even under the most opportune circumstances, as in our experiments, simple fluid replacement by isotonic crystalloid solution does not always prevent the development of the earlier stages of shock, and may not prevent the further progression, or avoid the irreversible stage of shock with fatal outcome.

3. Saline solution administered early is more effective than glucose solution as a preventive of shock and its progression.

4. The beneficial effect of saline solution must depend upon an action by Na^+ , Cl^- , or NaCl , independent of its restorative effect in replacing fluid lost from the blood.

While we are in no position to explain the mode of beneficial action of NaCl , which is apparently in addition to fluid replacement, several possibilities suggest themselves. Its action may be by virtue of the Na ion which may operate in several ways:

1. It has been shown that Na is a dilator of blood vessels (Roy, referred to in (10) (11)). Thus, it may lessen vasoconstriction which occurs as a concomitant of shock (12) and which is part of a vicious mechanism leading to the aggravation of tissue anoxia.

2. By virtue of the inverse relationship between sodium and potassium levels, a high sodium level would tend to lower the high potassium concentration reported to occur in experimental shock (11) or at least counteract its deleterious effects.

On the other hand, the chloride ion may lessen the acidemia which occurs in shock (cf. 1) by facilitating the production and excretion of NH_3 by the kidney in the form of the acid salt NH_4Cl . It may lessen chloride depletion accompanying vomiting which occurs in these dogs when they drink water. It may also be

that Cl facilitates oxygen transfer to the tissues and defers the condition of impending tissue anoxia (13).

It is also possible that several of these factors function in combination, and that the action is not due to either ion but to the NaCl molecule, e.g.: the possibility that NaCl lessens the tendency to dehydration by its water retaining effect. It is well established that NaCl is one of the best means of augmenting the extracellular fluid compartment of the body (14). This would avoid the deleterious effects of dehydration which in the venous occlusion experiments seems to play an important rôle in leading to the development of shock.

The action of NaCl in our experiments coincides with the effect of desoxycorticosterone acetate (DCA). We have shown that DCA given in divided doses, of about 6 mgm. per kilo of body weight, starting 24 hours before the operation and continuing for the first 24 postoperative hours, also prevented the development of irreversible shock in 73 per cent of the animals (8). Remington et al. (15) were unable to confirm this latter result but this may have been due to the fact that these investigators did not permit their dogs to have water, whereas our animals were allowed to drink at will. We observed that the dogs receiving DCA drank more freely than the untreated dogs, and did not vomit. Aside from maintaining high Na levels, the beneficial action of DCA could therefore have been due to the augmentation of the circulating blood volume and fluid reserves in a manner similar to that following infusion of isotonic NaCl.

Our experience indicates clearly that glucose is not as efficacious a crystalloid as saline. Since the glucose commonly administered in shock is made with saline the benefit falsely accredited to glucose may have been due to saline. Even when plasma and whole blood are given, there is, in addition to the proteins and red blood cells, an isotonic crystalloid content including NaCl, which latter may play an important rôle in the benefits of these blood volume replacers.

The prevention of dehydration by fluid replacement is one of the more important factors of shock therapy. As is evident from our study, intravenous injections of isotonic saline serves this purpose well, especially in the early stages in which capillary impairment is relatively non-existent. It may be valuable in any form of dehydration, and in massive vein thrombosis which this experimental method imitates. The fact that the *early* administration of isotonic saline is of such striking benefit in massive venous occlusion to such an extent that it actually prevents the development of irreversible shock and death, leads us to suggest that its trial in other forms of shock should be attempted more extensively than recently has been customary. If our results with the venous occlusion method are applicable to other forms of shock, and the recent studies of Rosenthal (16) (about which we learned when our studies were completed) show that isotonic saline is an effective agent in burn shock, it is suggested that in combat areas intravenous isotonic saline may be of value. Thus, when the supply of plasma or whole blood is limited, considerable benefit may be derived from the use of isotonic saline solutions in those injured individuals who cannot retain fluid by mouth, who are not greatly exsanguinated, and who are in the pre-shock or early shock stage. The limited supply of whole blood and plasma

available could thus be conserved for more urgent cases. It is not unlikely that the widespread use of isotonic saline solutions shortly after injury will actually lessen the need of plasma and other colloid solutions, since it may check the progress of the chain of events leading to shock. In those cases in which saline fails, recourse may still be had to plasma and whole blood (17). Emphasis is laid upon this point in an attempt to counteract the contemporary trend of indiscriminate utilization of plasma and whole blood and of concentrated forms of plasma and colloid solutions. There is a definite rôle for isotonic saline solution in conditions of dehydration and in states of injury without exsanguination in which the total quantity of plasma protein is not markedly reduced. The chief provision in the use of isotonic saline solution is that it be used early enough, then if it fails to be beneficial, whole blood, unconcentrated plasma or appropriate colloid solutions should be substituted.

SUMMARY AND CONCLUSIONS

1. The efficacy of isotonic sodium chloride and of glucose solution in prevention of the shock syndrome produced by venous occlusion of the hind limb was investigated. Sodium chloride was found to be of more beneficial therapeutic action than glucose, demonstrating that the simple addition of fluid per se is not the sole important factor in preventing this type of shock. The possible beneficial mechanisms of action of sodium chloride are discussed.

2. Our results demonstrate that isotonic saline solution if administered before alteration of capillary permeability has taken place, has a definite place as a therapeutic agent in the treatment of this and possibly other kinds of shock. Its early administration, when other types of blood volume augmentors are precious or not available, cannot be overemphasized.

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STUDIES ON THE PHYSIOLOGY OF MANGANESE IN THE RAT

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Indications for the necessity of manganese in animal nutrition originally were presented in 1926 by McHargue (1) but the first real evidence of a requirement awaited the work of Kemmerer (2), who employed mice on a mineralized diet. Testicular degeneration, impaired estrous cycle, and decreased viability of the young have been reported in rats as early symptoms of a manganese deficiency (3); improved techniques have resulted in also demonstrating impaired growth (4, 5). Lameness in pigs (6), sterility in cows (7) and perosis in chicks (8) likewise have been traced to a lack of this element.

Despite the evident needs for manganese, almost nothing is known of its rôle in the body, and little has been published on its physiology.

In the work done on chicks it was found (9, 10) that perotic chicks have lower blood and bone phosphatase activity than non-perotic chicks; in rats, liver arginase activity is considerably lowered in Mn-deficient animals (4, 5). Both arginase and phosphatase as well as several intestinal peptidases (11, 12) are activated by manganese—but in all of these cases the effect is not specific. There are several reports, also, on the rôle of manganese in connection with certain of the vitamins (13–15), but no confirmation of these findings has been forthcoming.

The problem of bone formation has presented itself in reference to the researches with chicks. Two cases of malformed bones also have been reported by Barnes et al. (16) as having occurred in rats raised on a low-manganese milk ration. The type of malformation noted was unusual and they attached some significance to this fact, although bone studies by Boyer et al. (4) failed to produce a similar picture on an obviously more deficient diet.

Thus it appears that a great deal of investigation remains to be done in this field. The very fact that growth is definitely impaired by a deficiency of manganese points towards some specific physiological rôle of this element in nutrition.

In this paper analytical determinations on manganese-deficient animals were made for the purpose of arriving at gross biochemical changes, which then might be used to indicate the fundamental processes in which manganese is involved. Blood, bone, enzymatic, metabolic, and endocrinal studies were carried out, and the results and interpretations of these results are reported in detail.

EXPERIMENTAL RESULTS. *Growth.* All animals used in this work were removed from breeding cages at two weeks of age, and placed with their mothers on the experimental ration until weaned one week later. This procedure prevented undue contamination of the young, and facilitated the production of a deficiency.

Previous work with chicks (9) and the recent investigations of Shils and

McCollum (5) indicate that high Ca and P ingestion produce more marked Mn-deficiency symptoms. In our work the basal ration used, designated as M-1, had a Ca/P ratio of 1.4; M-2 ration a Ca/P ratio of 0.85; M-3 ration a ratio of Ca/P of 2.55. The ingredients of these rations are summarized in table 1. Purification consisted of recrystallizing the sucrose once from a 60 per cent alcohol solution; use of reagent salts in all cases, with the $\text{Ca}_3(\text{PO}_4)_2$ being further purified by reprecipitation and the iron salts being made by dissolving reagent iron wire in concentrated hydrochloric acid. The casein was a vitamin-free product called Labco casein. Control animals were supplemented orally with 50 γ per day of manganese as the sulfate. The growth records reported in table 2 represent data obtained on these rations after a two week depletion period following weaning.

Table 2 clearly shows the differences in growth evidenced in a manganese deficiency. Although the figures for the M-2 ration indicate the slower onset of a deficiency as compared to the basal ration, the table unfortunately does not show the relatively more severe symptoms obtained on the higher Ca/P ratio with an earlier plateau in weight, and poorer efficiency. This was more readily apparent when paired-fed animals were used, a less efficient conversion of food to body weight being noted in all of the manganese-deficient animals as compared to their manganese-supplemented litter-mates.

In general appearance the manganese-low rats show signs of progressive emaciation, a poor and dirty hair coat, and instability. No paralysis, as reported by Shils et al. (5), was noted in animals maintained on the basal ration for as long as six months, although a noticeable weakness of the hind legs caused a decidedly impaired gait. A typically deficient animal is pictured in figure 1 beside a normal littermate.

Basal metabolism. Using published techniques (17), the respiration quotient and the basal metabolic rate in terms of calories/square meter/24 hours were obtained. No differences were found in R.Q.—all animals averaging about 0.75. Although a slight increase in B.M.R. was found in the deficient animals (1069 ± 30 compared to a control value of 990 ± 33), it is doubtful if much significance can be placed on this finding in view of the natural variation which would be expected.

Nitrogen metabolism. A significantly disturbed function of arginase activity in the animal might be expected to cause impaired nitrogen metabolism. Use of a milk ration in one case, and the synthetic basal ration with protein removed in another, showed no essential differences in feces excretion, urinary excretion, or retention of nitrogen in animals in intermediate stages of deficiency.

Bone studies. An extensive study of bone formation was called for in view of the reported differences noted in blood and bone phosphatase activity in chicks. The results of analyses of the femurs for length, volume (determined by the volume displacement of the bone in water), percentage of dry matter, percentage of ash on the dry ether extracted bones, and the density of the ash in the bone, are summarized in table 3. It is obvious from these data that the process of bone formation is significantly disturbed in the manganese-deficient animal. Not

TABLE 1
Composition of Mn-low rations

	M-1	M-2	M-3
Sucrose (per cent).....	11.0	11.0	11.0
Casein (per cent).....	29.0	29.0	29.0
Lard (per cent).....	53.5	51.5	51.5
Salts* (per cent).....	6.0	6.0	6.0
Na ₂ HPO ₄ ·12H ₂ O (per cent)....		2.0	
CaCl ₂ ·2H ₂ O (per cent).....			2.0
Vitamins:			
choline (per cent).....	0.5	0.5	0.5
p-aminobenzoic acid.....	200 mgm./kilo	200 mgm./kilo	200 mgm./kilo
flavin.....	10 mgm./kilo	10 mgm./kilo	10 mgm./kilo
pantothenic acid.....	25 mgm./kilo	25 mgm./kilo	25 mgm./kilo
B ₁	2 mgm./kilo	2 mgm./kilo	2 mgm./kilo
B ₆	15 mgm./kilo	15 mgm./kilo	15 mgm./kilo
nicotinic acid.....	25 mgm./kilo	25 mgm./kilo	25 mgm./kilo
halibut liver oil.....	1 drop/week	1 drop/week	1 drop/week
*Composition of salts:			
MgSO ₄ ·7H ₂ O.....	8	8	8
NaCl.....	20	20	20
KCl.....	10	10	10
Ca ₃ (PO ₄) ₂	61	35	40
Na ₂ HPO ₄ ·12H ₂ O.....		26	
CaCl ₂ ·2H ₂ O.....			21
ZnSO ₄ 10.0	4M.....	1	1
FeCl ₂ ·4H ₂ O 79.6			
CuSO ₄ ·5H ₂ O 10.0			
KI 0.4			
Ca content (mgm./gram).....	14.2	8.14	18.15
P content (mgm./gram).....	9.64	9.61	7.12
Ca/P ratio.....	1.47	0.85	2.55
Mn content = γ/gram.....	0.90	0.92	0.93
Av. daily food intake (grams)...	5-6	5-6	5-6

TABLE 2
Growth records of rats on various Mn-low diets*

RATION	ADDED MANGANESE				MANGANESE-LOW			
	Sex	Number of animals	Average wt. gain per day	Range	Sex	Number of animals	Average wt. gain per day	Range
			grams	grams			grams	grams
M-1	♀	15	2.70	(2.1-3.3)	♀	17	1.61	(0.7-2.4)
	♂	7	3.28	(2.8-3.8)	♂	17	1.73	(0.3-2.9)
M-2	♀	3	2.87	(2.7-3.1)	♀	4	2.33	(2.1-2.6)
	♂	3	4.28	(3.9-4.6)	♂	3	2.89	(2.4-3.3)
M-3	♀	2	3.12	(2.9-3.3)	♀	3	1.57	(1.0-2.0)
	♂	1	3.10		♂	3	1.97	(1.5-2.2)

* ♀ average for 4 weeks; ♂ average for 6 weeks.

only are the percentages of dry matter and ash lower but the concentration of ash in the bone is considerably decreased. These figures are actually an analytical expression of an observed weakness of the bone generally. Examination of longitudinal sections of these bones shows in the young, normally growing rat a



Fig. 1. Manganese-deficient rat with normal litter mate. Animals are 6 months old ♀♀ and weigh 130 grams and 230 grams respectively.

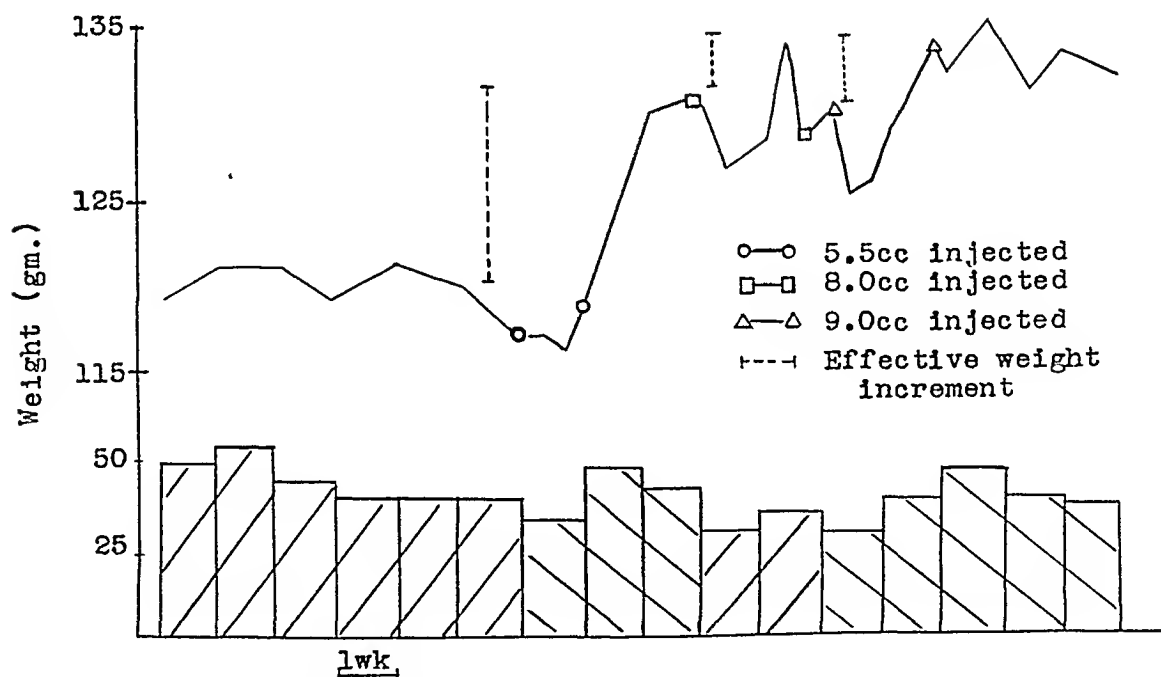


Fig. 2. Pituitary studies on Mn-deficient ♀ rat on M-3 ration. The top curve represents growth from 63 days of age; the bottom graph represents weekly food consumption.

somewhat spongy layer of trabeculae beneath the epiphyseal line in the head of the diaphysis. These trabeculae, however, are absent in the manganese-deficient animal—indicating a greater resorption of bone.

Although the bone ash is decreased there is no apparent difference in the Ca

and P constituents of the ash: the normal animals having 37.1 per cent Ca and 17.5 per cent P, and the deficient animals 37.0 per cent Ca and 18.0 per cent P.

Likewise, there is no change in the amount of Ca and P in the blood serum. Analyses of over thirty animals on all three experimental rations indicate a P content of 9.10 mgm./100 cc. for controls, as compared to 9.51 mgm./100 cc. for deficient animals; the Ca content is 11.87 mgm./100 cc. as compared to 11.58 mgm./100 cc. for the M-1 and M-2 rations, and about 2 mgm./100 cc. higher for the M-3, high Ca ration.

Balance studies on paired fed animals show some impairment of phosphorus absorption in manganese-deficient animals, but it is questionable if this accounts

TABLE 3
Summary of bone studies

RATION	AGE	- Mn				+ Mn			
		Bone length	Dry bone	Bone ash	Density	Bone length	Dry bone	Bone ash	Density
	days	cm.	per cent	per cent	ash/cc.	cm.	per cent	per cent	ash/cc.
M-1	67-80		55.4 \pm 3.4	58.3 \pm 3.2	0.42 \pm 0.05		59.1 \pm 0.7	62.3 \pm 0.6	0.51 \pm 0.01
	Diff.		-6.3%	-6.4%	-17.6%				
	82-129	\bar{x} = 2.87 \pm 0.7 σ^2 = 2.87 \pm 0.6	58.3 \pm 3.0	61.1 \pm 1.4	0.47 \pm 0.04	\bar{x} = 3.09 \pm .05 σ^2 = 3.51 \pm 0.12	64.2 \pm 0.7	64.6 \pm 0.5	0.57 \pm .02
	Diff.		-9.2%	-5.4%	-17.6%				
	130 and over	\bar{x} = 3.17 σ^2 = 3.23 \pm .04	63.3 \pm 1.2	63.4 \pm 0.8	0.56 \pm .03	\bar{x} = 3.38 \pm 0.10 σ^2 = 3.65	67.3 \pm 1.2	66.1 \pm 0.5	0.65 \pm .02
	Diff.		-5.9%	-4.1%	-13.8%				
M-2	82-129	\bar{x} = 3.29 σ^2 = 3.41	64.7 \pm 2.0	63.2 \pm 1.0	0.57 \pm .02	\bar{x} = 3.36 σ^2 = 3.83	66.7 \pm 0.7	65.2 \pm 0.4	0.66 \pm .00
	Diff.		-3.0%	-3.1%	-13.6%				
	130 and over	\bar{x} = 3.20 \pm 0.13 σ^2 = 3.55	63.8 \pm 0.7	63.3 \pm 0.7	0.58 \pm .03	\bar{x} = 3.40 σ^2 = 3.95	67.6 \pm 1.2	65.8 \pm 0.6	0.66 \pm .03
	Diff.		-5.6%	-3.8%	-12.1%				
	130 and over	\bar{x} = 2.91 σ^2 = 3.15	62.9 \pm 1.0	63.3 \pm 0.5	0.55 \pm 0.01	\bar{x} = 3.42 σ^2 = 3.75	69.8 \pm 0.6	66.6 \pm 0.5	0.70 \pm .03
	Diff.		-9.9%	-5.0%	-21.4%				

for the more severe symptoms evidenced on a high Ca/P diet—the absorption that does take place appears more than sufficient as noted by the amount of urinary P excretion, and body retention. The differences obtained are too small to claim a lack of phosphorus as the cause of impaired growth. Possibly the altered ration interferes somewhat with absorption of manganese in the intestinal tract, as has been reported in the case of iron (18).

Phosphatase. In view of the changes obtained in the bone, and the phosphatase work reported on chicks, a study of phosphatase activity in the rat was undertaken. Using the method of Bodansky (19), the activities of 0.1 cc. of plasma and 0.2 cc. of a suspension containing 5 per cent of bone (femur) in water

was measured. Activity was based on phosphatase units, expressed as milligrams of phosphorus (20) liberated in 1 hour at 37.5°C. from a sodium glycerophosphate solution buffered at a slightly alkaline pH. Values based on units per gram of green bone, and 10 cc. of plasma are given in table 4.

Contrary to the findings in chicks, the manganese deficient rats showed an increased phosphatase activity in the blood, although a slightly diminished

TABLE 4
Blood and bone phosphatase

RATION	- Mn			+ Mn		
	Sex	Phosphatase activity		Sex	Phosphatase activity	
		Blood	Bone		Blood	Bone
Age: 115 days						
M-1	♀	units/10 cc. 13.7	units/gram	♀	units/10 cc. 6.8	units/gram
	♂	20.2		♀	6.8	19.25
	♀	23.9	9.57			
	♂	19.5	9.39			
M-2	♀	15.8	17.59	♀	12.9	14.54
	♀	15.3	18.11			
M-3	♂	18.2	6.51	♀	5.6	11.18
	♀		13.56			
Average.....		18.1 ±2.7	12.46 ±3.97		8.0 ±2.4	14.99 ±2.83
Diff. from normal per cent.....		+125	-17			
Age: 150 days						
M-1	♀	12.4		♀	6.3	22.05
				♀	5.7	
M-2	♀	9.1	7.69	♀	5.4	12.51
	♂	7.8	13.40	♂	5.6	11.56
M-3	♂	7.6	10.43	♂	4.8	9.02
	♀	10.3	10.49	♀	5.6	17.84
Average.....		9.4 ±1.5	10.50 ±1.45		5.6 ±0.3	14.60 ±4.28
Diff. from normal per cent.....		+68	-28			

activity in the bone. This latter finding, however, cannot be termed significant due to the wide range of activities noted in various samples, and the overlapping of results. It would appear that there is no change in the phosphatase of the bone.

The higher values obtained in the blood of younger deficient rats is probably an expression of the retarded development of these animals—very young rats

have a much higher phosphatase in the blood than older ones. The weakness of the bone, too, may cause a leakage of phosphatase into the blood as is believed to occur in rickets.

Hemoglobin. With the general weakness observed in the bones of deficient animals one might suspect some interference with the formation of hemoglobin which takes place in this organ. Studies made on the blood of rats at different ages do indicate a gradually increasing difference between the values obtained for the deficient animals as compared to the controls. These results are summarized in table 5. In an attempt to determine if these differences are caused by some disruption in the production of hemoglobin, regeneration studies were also carried out. This was done on paired fed animals by removing an amount of blood approximately 25 per cent of the total (considered to be 10 per cent of the body weight). The findings reported in table 6 seem to indicate some trouble in this respect, in manganese-deficient animals.

TABLE 5
Hb values at different ages

AGE	+ Mn		- Mn		DIFFER- ENCE
	Number of animals	Hemoglobin	Number of animals	Hemoglobin	
		grams/per cent		grams/per cent	
<i>wks.</i>					
5	4	13.55 (13.2-13.8)	4	12.56 (11.6-13.6)	-0.99
6	10	14.24 (13.8-15.2)	10	13.06 (12.0-14.4)	-1.08
9	7	14.71 (13.3-16.7)	7	13.41 (12.2-14.2)	-1.30
10	8	15.23 (14.6-16.3)	8	14.17 (12.3-14.9)	-1.06
12	6	15.43 (14.7-15.9)	6	13.40 (11.8-14.4)	-2.03
15	5	15.63 (15.2-16.1)	8	14.03 (13.7-14.6)	-1.60
19	6	15.38 (14.6-15.9)	8	12.71 (11.2-13.7)	-2.67

Pituitary studies. Orent and McCollum in early work on manganese deficiency in rats mentioned a possible relationship of Mn to pituitary function.

If manganese does play such a rôle in the body it would probably be in one or both of two functions; the production of the hormones, and/or the proper utilization of the hormones after secretion. If manganese is involved only in the production, then injections of suitable extracts of the pituitary should result in elimination of manganese-deficiency symptoms. As the manganese content of the pituitary is around 4 or 5 γ per gram, and water extracts contain only a slightly detectable quantity, contamination with manganese by means of small injections is negligible.

Suitable extracts of pituitary gland were made by homogenizing one part of minced, frozen sheep anterior pituitary gland with 2 parts of water and allowing the suspension to autolyze for two days at 0°C. The ground tissue was then centrifuged down, and the semi-clear water extract separated.

The animals used in these studies were all over 100 days old, and had exhibited signs of plateauing in weight for several weeks. Injections of the extract were

TABLE 6
Hemoglobin regeneration—paired feeding

AVERAGE WEEKLY FOOD CONSUMP- TION	+ Mn							- Mn				
	Sex	Age	Wt.	Hemo- globin	Blood removed		Hemo- globin regener- ated	Wt.	Hemo- globin	Blood removed		Hemo- globin regener- ated
gms.		days	gm.	gm./100 cc.	cc.	per cent	gm./100 cc.	gm.	gm./100 cc.	cc.	per cent	gm./100 cc.
37.6	♀	66	137	15.79	3.5	23.6		120	12.64	2.8	22.8	
				11.83			-3.96		10.09			-2.55
		73	148	14.67			+2.84	124	12.76			+2.67
		80	156	15.68	4.8	27.4	+3.85	122	12.21	3.6	25.5	+2.12
		100	171	16.57				136	12.06			
				12.14			-4.43		9.57			-2.49
		114	169	15.25			+3.11	133	12.91			+2.34
		121	170	16.69			+4.55	137	13.23			+3.66
39.3	♀	66	154	14.97	3.8	24.6		141	15.05	3.1	22.0	-2.84
				11.98			-2.99		12.21			
		73	155	14.86			+2.68	143	13.11			+0.90
		80	163	15.17	5.2	29.0	+3.15	144	14.16	3.8	24.8	+1.95
		100	172	16.22				153	14.78			
				11.71			-4.51		11.37			-3.41
		114	172	15.17			+3.46	147	13.77			+2.40
		121	178	16.57			+4.86	142	14.16			+2.79
34.0	♂	66	116	15.68	2.8	22.8		102				
				11.48			-4.20					
		73	121	11.28			-0.20	110				
		80	118	14.28	4.6	27.4	+2.80	114	14.47	3.1	24.8	
		100	166	14.28				125	14.78			
				10.43			-3.85		10.89			-3.89
		114	169	13.97			+3.54	116	13.89			+3.00
		121	168	15.60			+5.17	107	12.84			+1.95
36.6	♀	66	110	15.17	2.7	22.5		95	13.77	2.3	23.0	
				11.50			-3.67		10.66			-3.11
		73	117	14.28			+2.78	101	12.64			+1.98
		80	127	15.68	3.6	24.6	+4.18	104	13.89	2.2	20.0	+3.23
		100	150	16.80				115	13.97			
				13.24			-3.56		10.81			-3.16
		114	140	15.91			+2.67	108	9.26			-1.55
		121	139	15.91			+2.67	109	10.81			+0.0
Averages		66		15.40	3.2	23.4			13.82	2.7	22.6	
				11.70			-3.70		10.99			-2.83
		73		13.77			+2.07		12.84			+1.85
		80		15.20	4.6	27.1	+3.50		13.42	3.2	23.8	+2.43
		100		15.97					13.90			
				11.88			-4.09		10.66			-3.24
		114		15.08			+3.20		12.46			+1.80
		121		16.19			+4.31		12.76			+2.10

made subcutaneously in 0.25 cc. amounts, and were given regularly through the dosage period. Exemplary responses to these injections are shown in figure 2, along with a short control experiment. Weekly food consumption values are also given.

The effective weight increment is defined as the measured increase in weight over that which would be expected from the naturally exhibited growth of the animal as observed in a several weeks' preliminary period.

Effects noted above could not be attributed to adrenotropic action of the pituitary extract, as injections of 8.5 cc. of Adrenal Cortex Extract (Upjohn) over a ten day period in one manganese-deficient rat failed to cause change in weight or food consumption.

DISCUSSION. In the case of manganese deficiency, from the work reported here, and by others, the following positive facts are known for the rat.

1. In a mild deficiency growth is normal but the oestrous cycle of female rats is interfered with, and testicular degeneration occurs in males.

2. In a more severe manganese-deficiency the animals show definitely impaired growth, signs of emaciation, weakness of the limbs, poor hair coat and some nervousness.

3. Analyses of the bones indicate poorer bone formation in the deficient animal.

4. A slight decrease in the hemoglobin concentration of the blood is evidenced, and some interference with hemoglobin regeneration.

5. Contrary to the work with chicks an *increase* is noted in serum phosphatase.

6. The liver arginase activity is definitely decreased in the deficient animal.

7. A more aggravated deficiency may be obtained by raising the Ca/P ratio of the ration; a slightly decreased absorption of P is noted in the deficient animals.

8. In a limited number of cases injections of extracts of sheep anterior pituitary gland have resulted in a slight initial increase in weight, not obtained with succeeding injections.

Negatively there has been shown:

1. No difference in basal metabolic rate between normal and Mn deficient rats.

2. No gross differences observed in nitrogen absorption or excretion.

3. Bone phosphatase activity is unchanged in the rat (though lower in the chick).

4. No histological abnormalities detected in adrenal, kidney, pituitary and thyroid of manganese-deficient rats.

To the above might be added the *in vitro* studies linking manganese to several enzymes systems as a non-specific activator.

It appears evident from the data available that manganese functions in some rôle affecting the well-being of the entire body. Decreased food consumption evidently cannot be blamed for the results noted, as all findings were checked on paired fed animals. No one outstanding symptom as yet has been discovered. Lack of growth and impaired bone formation may be correlated; likewise trouble

with the bone may result in increased blood phosphatase (as in rickets) and deficient hemoglobin regeneration, and slight anemia. The effect on liver arginase has been noted, too, in cases of protein deficiency or impaired metabolism (21).

The possibility of manganese functioning in some connection with the pituitary gland has been offered. The rôle of the pituitary in reproduction and growth is generally known. On removing the gland a cessation of growth occurs which is localized in the growing epiphyseal cartilage (22). Hypophysectomy also has been shown (23) to cause a slight drop in the hemoglobin value; a decrease has been reported, too, (24) in liver arginase activity. A correlation between a manganese-deficiency and these findings is evident.

SUMMARY

1. Synthetic rations with varying Ca/P ratios were made which would provide only 5 γ per day of manganese.

2. Rats raised on the experimental rations exhibited impaired growth, more pronounced in cases where a high Ca/P ratio was present.

3. Manganese deficient rats had poorer bone formation, averaging about 5 per cent less ash, and 15 per cent less ash per unit volume; Ca and P constituents of the ash were unchanged.

4. A slight progressive anemia was noted in the deficient animals, with values averaging about 2 grams per 100 cc. of blood below normal. Hemoglobin regeneration was decreased.

5. Paired feeding trials showed the same differences in growth, bone, and hemoglobin as in animals fed ad lib.

6. No differences in bone phosphatases were found, but a two to three-fold increase in blood serum phosphatase was present.

7. Values for Ca and P of the blood were normal, though a slight decrease in P absorption was noted in deficient animals.

8. No differences could be found in nitrogen metabolism or basal metabolic rate.

9. Anterior pituitary extracts have been shown to have some effect in stimulating growth of deficient animals for a short period.

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THE PERIPHERAL VISUAL ACUITY OF 100 SUBJECTS¹

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Acuity curves for peripheral vision are well known, those of Wertheim (3) and Koester (1) being frequently reproduced. Since their findings nearly fifty years ago the subject has been investigated by numerous workers whose results have been reviewed by Traquair (2) who lists the data of some 28 investigations by 11 workers. Although their methods were essentially the same the results differed considerably. Not a great many subjects were examined. This suggested a more or less wide variation of peripheral visual acuity under normal conditions. Therefore this investigation undertook the development of a suitable test for peripheral visual acuity and the testing of a sufficient number of individuals to determine the extent of the suspected variation.

APPARATUS. Tests were run on a 25 cm. perimeter (American Optical Company—AO 460). The following apparatus was built:

1. *Illuminator.* A 60 w. Mazda daylight lamp was fixed on the locus of all points equidistant from points along the perimeter arms, 25 cm. from the subject's eye. The light turned with the perimeter arms. The subject's eye was protected from glare by a shade. A 75 mm. square of white bristol board was attached to the rod supporting the lamp, near the bulb, placed so as to throw diffused light toward the eye being tested.

2. *Target carrier.* A carrier for the targets was made of 3 thicknesses of bristol board with the following dimensions: (a) front card; 75 mm. sq., 55 mm. cutout in center; faced with photographic paper; (b) middle card; 75 mm. sq., on three sides; all sides 5 mm. wide; built up with photographic paper for thickness; (c) back card; 75 mm. sq.; screw head in center flush with card; semicircular cutout of 10 mm. radius in middle of top edge. The carrier was fastened to the test object carriage so that it would revolve freely around its center.

3. *Targets and test objects.* The targets of bristol board and photographic paper were cut 64 by 69 mm. to fit in the carrier. The test objects were Landolt Broken Circles (diameter equals five times width of line equals width of break) photographed and printed on the targets in the following sizes: width of break in mm. $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, $2\frac{1}{2}$, 3, 4, 5, 6, 7, 8, 9, 10. The center of the test object coincided with the center of the carrier.

4. *Identification button.* A circle (10 mm. radius) of white bristol board with a hole in the center was fitted on the button on which the subject fixed his eye. The numbers I, II, III, IIII were marked on this circle in positions corresponding

¹ The cost of the statistical analyses here reported was met by the Smith Research Fund of the University of North Carolina.

to the points of the compass. The subject referred these numbers to the four possible positions of the break in the test object, signaling with a buzzer.

5. *Booth.* Black, non-reflecting cloth was draped wherever the subject's visual field extended. All visible apparatus except the carrier, target, and identification button were black.

6. *Blindfold.* A one-eye blindfold was used to cover the eye not being tested.

7. *Operator.* The operator wore a black robe and black gloves. He was equipped with a black paddle large enough to conceal the carrier during changes.

PROCEDURE. Nine points on the temporal periphery of each eye were tested on meridians 45 degrees apart. The following points were tested on the meridians and angular deviations indicated: out 30°, 60°, 90°; down and out 30°, 60°, 90°; down 30°, up 30°, and up and out 30°, in the order mentioned. With the room blacked out except for the illuminator on the instrument, the subject was seated at the perimeter and a large size target was inserted in the carrier set at the first point to be tested. It was adjusted in one of the four possible positions while covered with a black paddle and then revealed to the subject who signalled an answer. If the subject could not tell he was made to guess. The target was covered again and the position of the break changed. This procedure was repeated until the subject got four consecutive correct answers, or two misses (not necessarily consecutive). When the subject succeeded the next smallest size test object was used. This was continued until the subject failed. The smallest test object successfully identified was scored, using the width of the break of the test object in millimeters to denote the score. During identification the eye not being tested was blacked out. Fixation was checked by watching the subject's eye.

For purposes of correlation the peripheral test was preceded by a standard test for central acuity (Snellen illuminated chart—metric measurement) and the Ishihara color test. The wheel test for astigmatism was given on the uncorrected eye. Age and sex were recorded.

One hundred subjects selected at random were tested as described above. The peripheral test took from 40–60 minutes. The left eye was always tested first, the subject resting about 5 minutes between eyes. Twenty of the original group were retested under identical conditions after a lapse of about 2 to 3 months.

RESULTS. It was found that the peripheral acuity was so weak at the 90° points that from 89–94 of the 100 subjects could not identify the largest target used (10 mm.). These points were discarded in calculation of the total score of each individual, regardless of performance. Since the score recorded was the size of the break of the smallest test object correctly identified, the actual acuity lay somewhere between that value and the next smallest size. The midpoint between the two was chosen as being presumably more accurate. The sum of the individual scores for 14 points was chosen as the total score for that individual. The smaller the value the better was the acuity. Therefore the reciprocal of this figure was chosen as the value from which percentages were calculated.

The means and standard deviations for the group of 100 are shown in the black boxes of figure 1. Among the 100 subjects there were 30 who would have qualified as aviation cadets on the basis of age, sex, and visual requirements. They were males 18 to 27 years of age inclusive, with normal or better central vision, normal color vision, and no astigmatism. Their means and standard deviations are shown in the white boxes in figure 1. As a whole their scores were better but the degree of variation among individuals, which the height of the standard deviation box indicates in contracted form, is nearly as great as that of the whole group. On the basis of total scores the 2nd and 99th in degree

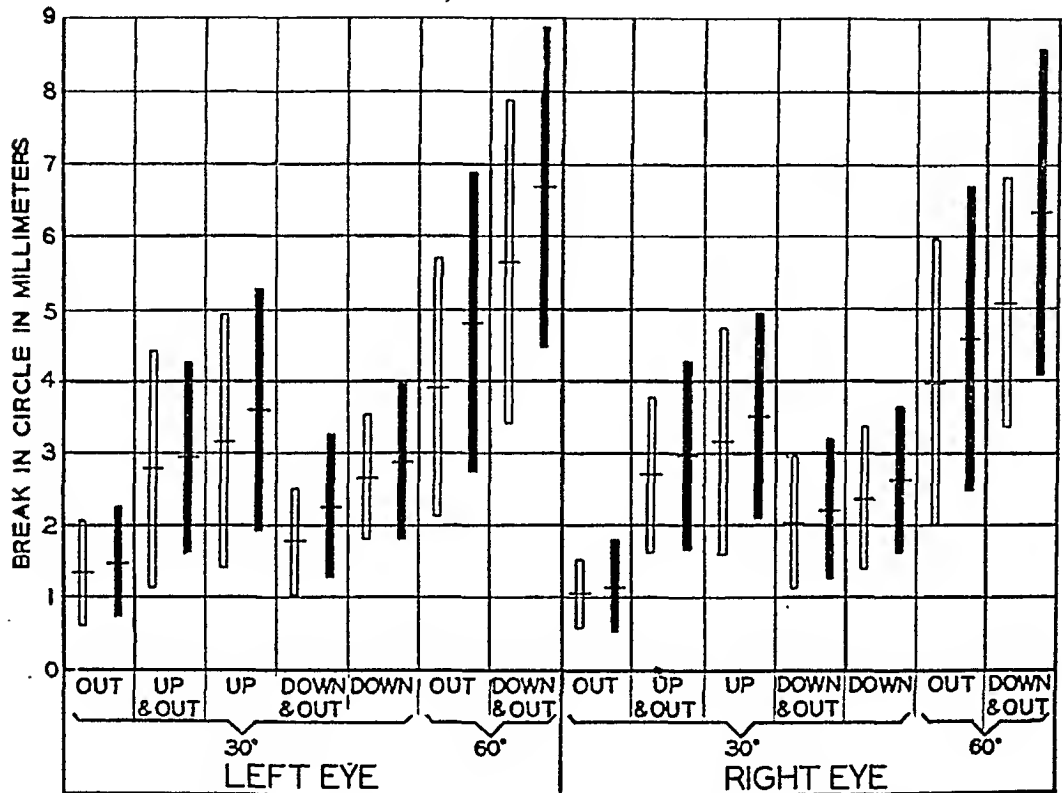


Fig. 1. Graphical representation of means (averages) and standard deviations of peripheral visual acuity. Boxes show limits of standard deviations, cross bars are means. Black boxes are total group of 100. White boxes are selected group of 30 (males 18-27 incl., normal or better central acuity, normal color vision, no astigmatism).

of peripheral acuity were members of this selected group of 30. The actual spread of the total scores of both groups is recorded in table 1. In the group of 100 the reciprocal of the average score was arbitrarily chosen to represent 100 per cent and the value for each individual or group expressed by a comparative percentage. In the test-retest group of 20 individuals the original score, individual or group average, was regarded as 100 per cent and the second score expressed in comparative percentage. Of this group the best subject improved about 50 per cent and the worst declined 8 per cent.

Table 2 shows the relationship of peripheral visual acuity to other measured data. Men scored 11 per cent better than women. The varying average scores

for the different age groups show that there is not necessarily a diminution with advancing years. The eight color blind individuals were 8 per cent below par. The deficiency is somewhat minimized because the best subject, scoring 364 per cent, was a member of this group.

TABLE 1
Averages and actual limits; test-retest scores

GROUP	100	BEST	WORST	SELECTED 30*	BEST	WORST	20 TEST-RETEST	
							First	Second
Score.....	47.90	13.16	110.90	41.66	22.81	92.20	50.07	42.17
Reciprocal.....	0.02088	0.07599	0.00902	0.02400	0.04384	0.01085	0.01997	0.02371
Percentage.....	100	364	43	115	210	52	100	116

* Males 18-27 yrs. old inclusive, normal or better central vision, normal color vision, no astigmatism.

TABLE 2
Correlation of peripheral visual acuity scores with other data

GROUP	SEX		AGE				COLOR VISION
	Males No. = 87	Females 13	17-20 13	20-29 67	30-39 13	40-70 7	Color blind 8*
Score.....	47.16	52.88	41.58	49.21	48.62	45.82	51.86
Reciprocal.....	0.02120	0.01891	0.02405	0.02032	0.02057	0.02182	0.01928
Percentage.....	102	91	115	97	99	105	92

* All male; 7 red-green type, 1 unclassified (blue green?).

TABLE 3
Correlation of central acuity with peripheral acuity in 200 eyes

GROUP	200	NO. = 92	19	19	10	9	12	12	4	23
		6/5	6/6	6/7.5	6/10	6/12	6/15	6/20	6/30	6/60*
Score.....	23.95	21.58	20.63	26.61	25.53	21.94	26.22	28.98	25.73	29.93
Reciprocal..	0.04175	0.04633	0.04853	0.03757	0.03917	0.04558	0.03822	0.03451	0.03887	0.03341
Percentage..	100	111	116	90	94	109	92	83	93	80

* Some eyes weaker than 6/60.

Table 3 shows the relation of peripheral to central acuity. The same method of comparative percentages has been used but was calculated for each eye separately. The degree of correlation between the two values (Pearson product-moment correlation) was found to be positive at 0.38, a figure too low to yield any practical reciprocal predictive value.

DISCUSSION. The variation of peripheral visual acuity among individuals was found to be even greater than anticipated. Table 1 shows the best subject

to possess peripheral acuity about $3\frac{1}{2}$ times that of normal and about $8\frac{1}{2}$ times that of the weakest subject.

The relative average acuity for each point as noted in figure 1 agrees in the main with the curves of Wertheim (3). The actual variation of acuity for each individual point is of course greater than the limits of the standard deviation boxes. For example, on the up 30° point, the smallest test object identified was size 1, while 2 of the subjects could not identify size 10, the largest used. On each 60° point some individuals failed on size 10.

It is notable that the variation of the acuity for any point increased as the acuity itself decreased. This is illustrated by the fact that the higher the mean in figure 1, the larger the standard deviation box.

The data reported in table 3 indicate that central acuity is not a reliable indicator of peripheral visual acuity. This is in accord with the fact that the focusing power of the lens, which is largely responsible for central acuity, hardly functions at all beyond 30° from the line of vision. Also the failure of peripheral vision to decline regularly with advancing age as indicated in table 2 is understandable, since the decline in central vision is due to lens changes. The lack of high grade positive correlation between peripheral visual acuity and other measured factors seems to justify the statement that peripheral visual acuity is an independent visual function.

Especially interesting is the result of the test-retest experiment on 20 subjects. This technique was intended for use as a check on the reliability of the test, but the subjects, with very few exceptions, did better on the retest. Despite this improvement the scores for the two eyes of any one individual remained very close together. It was decided to check the reliability of the test on the assumption that, for any one subject, the score of one eye ought to be exactly the same as the score of the other. Calculation on this basis (Pearson product-moment correlation, corrected by Spearman-Brown formula) yielded a reliability of 0.91, a figure well within the allowable limits of variation for such a test. The improvement on the test-retest technique was interpreted to be bona fide improvement in peripheral acuity due to the practice afforded during the two tests, each of which lasted 40 to 60 minutes. This interpretation is supported by the fact that the right eye, which was always the second tested, scored better than the left. In a series of tests (not reported here) in which the order of testing the eyes was reversed, the second eye tested (left) showed better scores than the first. These indications that simple practice can train peripheral acuity are the basis of further investigations now in progress.

SUMMARY

1. A new test for peripheral visual acuity is described.
2. One hundred subjects have been tested.
3. Peripheral visual acuity is a very variable value, the total scores running from 43 per cent to 364 per cent of the average for the whole group.
4. Peripheral visual acuity is an independent visual function.

5. The weaker the peripheral visual acuity for any point the greater is the variation for that point.

6. The collected evidence indicates that peripheral visual acuity can be trained.

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THE PRODUCTION OF HYPOTHALAMIC OBESITY IN RATS ALREADY DISPLAYING CHRONIC HYPOPITUITARISM¹

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Although the early literature on experimental obesity abounds in reports claiming the production of obesity after various operative procedures on the hypophysis of several species, few research workers in recent years have stressed the importance of hypophysial damage as the actual cause of the syndrome in laboratory animals. In all probability, the observed outcome of the early experiments was the result of coincidental injury to the tuber cinereum. At any rate, it is certainly true that during the last 20 years numerous workers have removed the hypophysis in whole or in part from large numbers of animals without inducing adiposity. (Brown, 1923; Smith, 1930; White, 1933; McPhail, 1935; Lee and Ayres, 1936; Reiss, Epstein and Gothe, 1937; Dandy and Reichert, 1938; Phillips and Gilder, 1940; Winternitz and Waters, 1940; Hetherington and Weil, 1940; Hart and Magiday, 1941.) As Newburgh (1942) has hinted, there may indeed be no such disorder as hypophysial obesity—or if there is, there has been no convincing demonstration of it. Sevringhaus (1940) likewise seems inclined to this opinion, at least insofar as the so-called “hypopituitary” forms are concerned.

Previous work from this laboratory (Hetherington and Ranson, 1940) having demonstrated that a high degree of obesity can invariably be obtained in rats by the making of certain bilateral hypothalamic lesions, an attempt was later made to determine whether the hypothalamic destruction produced its effect through the hypophysis. Such a situation, even though it placed the hypophysis in a subordinate rôle, would still vindicate partially the view that dysfunction of the pituitary may co-operate to bring about the obesity. It was found (Hetherington and Ranson, 1942) that total hypophysectomy one week after the making of the hypothalamic lesions did not in any way impede the appearance of the adiposity, which was as pronounced as in cases with intact hypophyses. Hypothalamic lesions, therefore, do not cause excessive fat deposition by inducing some pathological imbalance of hypophysial secretions. One last possibility was evident: The disordered hypothalamic mechanism leading to obesity might be unable to exercise its influence in tissues which had undergone all the metabolic changes succeeding hypophysectomy and the thyroid and adrenal atrophy consequent thereto. On this supposition it was decided to repeat the experiment, using animals already long in a hypopituitary state before the making of the hypothalamic lesions.

Accordingly, a number of young male and female six-weeks-old rats were

¹ Aided by a grant from the Committee on Research in Endocrinology of the National Research Council.

hypophysectomized and observed and weighed at intervals for a period of 75 to 80 days. This is considerably longer than the interval required, according to Smith (1930) and Crooke and Gilmour (1938), for the attainment of maximum atrophy in the thyroids, adrenals, and gonads. If at the end of that time little or no increase in the body weight of the hypophysectomized rat had occurred, the gland ablation was assumed to be complete, and the rat was selected for the subsequent experiment. The hypothalamic lesions known to produce adiposity in the rat were made by a method which has already been described elsewhere (Clark, 1939; Hetherington and Ranson, 1940). Not including a number which died very shortly after the second operation (the hypophysectomized rat does not tolerate severe operative procedures very well), a total of 10 doubly operated animals was obtained. Several hypophysectomized and unoperated littermate controls were preserved. Aside from initial stays in a warm (28°C.) incubator and careful nursing the doubly operated animals were kept under the same conditions as our ordinary operated rats.

With the exception of two which died unexpectedly about two months after the second operation the rats were kept alive about 11 weeks. At the end of the experiment all the rest were anesthetized and measured, and then killed by decapitation. The base of the brain and the sella were sectioned together, stained with a combination of cresyl violet and eosin, and examined for remnants of hypophysial tissue. The sphenoid bone surrounding the original aperture made for hypophysectomy, together with some of the soft tissue ventral to it, was also preserved, decalcified, and studied histologically.

All the ages, post-operative times, initial weights, weight increases, and body lengths of the rats are shown in table 1. As may be seen none of the hypophysectomized rats showed any signs of putting on excessive weight during the 74 to 83 days following hypophysectomy. When, however, the 10 hypophysectomized rats subsequently received hypothalamic lesions they then became very fat. This obesity began to be quite apparent in most cases in a matter of 3 to 4 weeks, though a renewed tendency to gain weight rapidly was evident in all even before this time—as soon, in fact, as recovery from the second operation passed beyond the acute stage. The ratios of the cube roots of the animals' body weights in grams to their body lengths in centimeters (Lee, 1929) reveal that these rats became relatively just as fat as other animals previously reported, both those not hypophysectomized, and those hypophysectomized after placement of hypothalamic lesions. The ratios of the rats which were only hypophysectomized fall well within the range for normal rats; they usually, of course, display some decline in the size of the ratio from the pre-hypophysectomy level because of their loss of weight during the early stages.

The obese rats became rather irritable and unmanageable after the second operation, in marked contrast to their earlier pliant, submissive behavior after hypophysectomy. They retained, however, their soft white hair, though this in many instances became somewhat sparser. They did not display any tendency to resume skeletal growth. At autopsy the gonads, adrenals, and thyroid

were of course very small; no attempt was made to weigh these, or examine them histologically. There were in these obese dwarfs the usual large deposits of fat in all the normal depots which are seen in ordinary rats displaying hypothalamic obesity.

In the light of this evidence it seems altogether unlikely that the hypophysis may be considered to play any rôle of importance, either of an inhibitory or an augmentative nature, in hypothalamic obesity. Not only does removal of the hypophysis during the period of onset of the disorder fail to balk the excessive fat deposition, but even the pre-existence of a state of full-blown hypopituitarism

TABLE 1

Data on hypophysectomized rats in which hypothalamic lesions were made, and on their hypophysectomized and normal littermate controls

RAT NO.	TYPE OF OPERATION	HYPOPHYSECTOMY			HYPOTHALAMIC LESIONS MADE			AUTOPSY			
		Age	Weight	Nose-anus length	Age	Weight	Nose-anus length	Age	Weight	Nose-anus length	W ¹ /L.
		days	grams	cm.	days	grams	cm.	days	grams	cm.	
Rd-19♂	Pit.*	42	143	18.4				192	140	18.7	0.277
Rd-20♂	Pit. and Les.	42	142	18.0	122	155	18.3	191	251	18.2	0.347
Rd-21♂	Pit. and Les.	42	132	17.7	122	140	18.1	191	274	18.3	0.356
Rd-22♀	Pit. and Les.	42	130	17.7	122	130	17.7	191	283	17.9	0.365
Rd-23♀	Pit. and Les.	42	124	17.3	122	121	17.5	179	197	17.4	0.334
Rd-24♀	Pit.	43	125	17.2				192	128	17.5	0.288
Rd-25♀	Pit. and Les.	43	121	17.0	122	133	17.4	191	252	17.6	0.359
Rd-26♀	Pit. and Les.	43	115	17.3	126	112	17.0	191	205	17.2	0.343
Rd-27♀	Normal	43†	110†	16.8†				192	235	22.5	0.292
Rd-28♂	Pit. and Les.‡	42	162	19.2	116	177	18.9	186	340	20.3	0.344†
Rd-29♂	Pit. and Les.	42	150	18.5	116	160	18.0	186	265	18.9	0.339
Rd-30♂	Pit. and Les.	43	143	18.3	119	126	18.1	186	265	18.4	0.349
Rd-31♂	Pit.	43	143	18.4				187	147	18.8	0.281
Rd-32♂	Pit. and Les.	43	139	17.9	120	144	18.2	164	212	18.1	0.329
Rd-33♂	Normal	43†	142†	18.2†				187	485	25.7	0.305

* "Pit." indicates only a hypophysectomy was done. "Pit. and Les." indicates a hypophysectomy was followed about 11 weeks later by the making of hypothalamic lesions.

† This animal was normal. The data were taken at the time its litter mates were hypophysectomized.

‡ A very small fragment of anterior lobe tissue was found in histological sections of the sella.

does not interfere with appearance of the syndrome in just as marked a form as in rats in which the hypophysis is intact. Animals in which both operations have been performed present a picture of obesity superimposed upon pituitary dwarfism. These facts, taken together with the finding that obesity does not ensue when all or part of the hypophysis is removed without damage to the overlying brain, would appear to dispose of malfunction of the hypophysis as an explanation of the adiposity often associated with "pituitary regional disease" (Sevringhaus). It is the hypothalamic disability which is solely responsible for the obese condition.

SUMMARY

Ten rats hypophysectomized and observed for periods of about 11 weeks showed no signs of developing obesity. Some, even after so long a time, had not even attained their preoperative weight; others had surpassed it by a few grams only. They could be considered, therefore, to be in a state of chronic hypophysial insufficiency. Hypothalamic lesions were then produced in these animals by the Horsley-Clarke technic. Within 3 to 4 weeks they began to display rapid fat deposition, and 11 weeks after the second operation they were markedly obese. This evidence shows that the fat depots are still able to store excess fat in response to hypothalamic damage even after they have suffered the changes in tissue metabolism presumed to be attendant upon hypophysectomy. Since neither total nor partial hypophysectomy produces adiposity, or prevents its appearance after hypothalamic damage is done, it is not likely the hypophysis is involved in the production of obesity often associated with injury to structures in the pituitary region. Hypothalamic disorder appears to be the sole responsible factor.

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INHIBITION OF THE TURTLE'S ATRIA BY SINGLE INDUCTION SHOCKS APPLIED DIRECTLY¹

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A recent investigation of the inhibition produced by direct stimulation of the turtle's atria (1) demonstrated the selective inhibiting effect of brief electric currents (condenser discharges or induction shocks) applied repetitively. Further experiments employing atropine and eserine gave strong support to the conclusion that the inhibition was conditioned on the release of acetylcholine at vagus terminals. The ventricle which is not directly inhibited by the vagus was not inhibited by such direct stimulation. Within the range of intensities of current employed in this previous investigation, e.g., a Harvard inductorium activated by a $\frac{1}{2}$ to 3 volt primary current, any effect resulting from a single shock was always excitatory. Since a single vagal volley has been proved to be effective both for the mammalian heart (2) (3) and the heart of the turtle (4) (5), it has seemed desirable to test the effect of stronger single shocks applied directly to the atria.

EXPERIMENTAL. Turtles, 6-8 in. shell length, chiefly *Pseudemys elegans*, were used. The atria with attached sinus tissue were removed and the contractions of the double-atrial preparation were recorded on a kymograph by means of a light isotonic lever. The arrangement was such that the tissue could be immersed in solutions as desired. Single break shocks from a Harvard inductorium were applied by means of light flexible electrodes. In testing for inhibition it was the usual procedure to apply a break shock during the refractory period since in this way a premature beat and its accompanying compensatory pause were avoided and any depression could be measured more accurately.

Proceeding thus, it was found that when current intensity was increased sufficiently depression followed such single shocks. The inhibiting threshold for a single shock is much higher than for repetitive stimuli since these latter are summated and their effect is cumulative. With the Harvard inductorium employed, it was necessary to increase the voltage of the primary current to 5 or 6 volts before single shocks produced definite inhibition. This difference in threshold explains the previous inability to produce inhibition by single shocks applied directly. The depression is not an injury effect since within the range of stimuli employed recovery was prompt and complete.

At break shock values near the threshold for inhibition the depression was limited to decrease in amplitude (negative inotropic effect). With stronger shocks a negative chronotropic effect was added. These effects are illustrated in figure 1, A, B and C.

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The maximum reduction in strength was not immediate but increased progressively for two or more beats and then disappeared slowly, the total time of depression averaging between 30 seconds and 1 minute. In figure 2 a typical

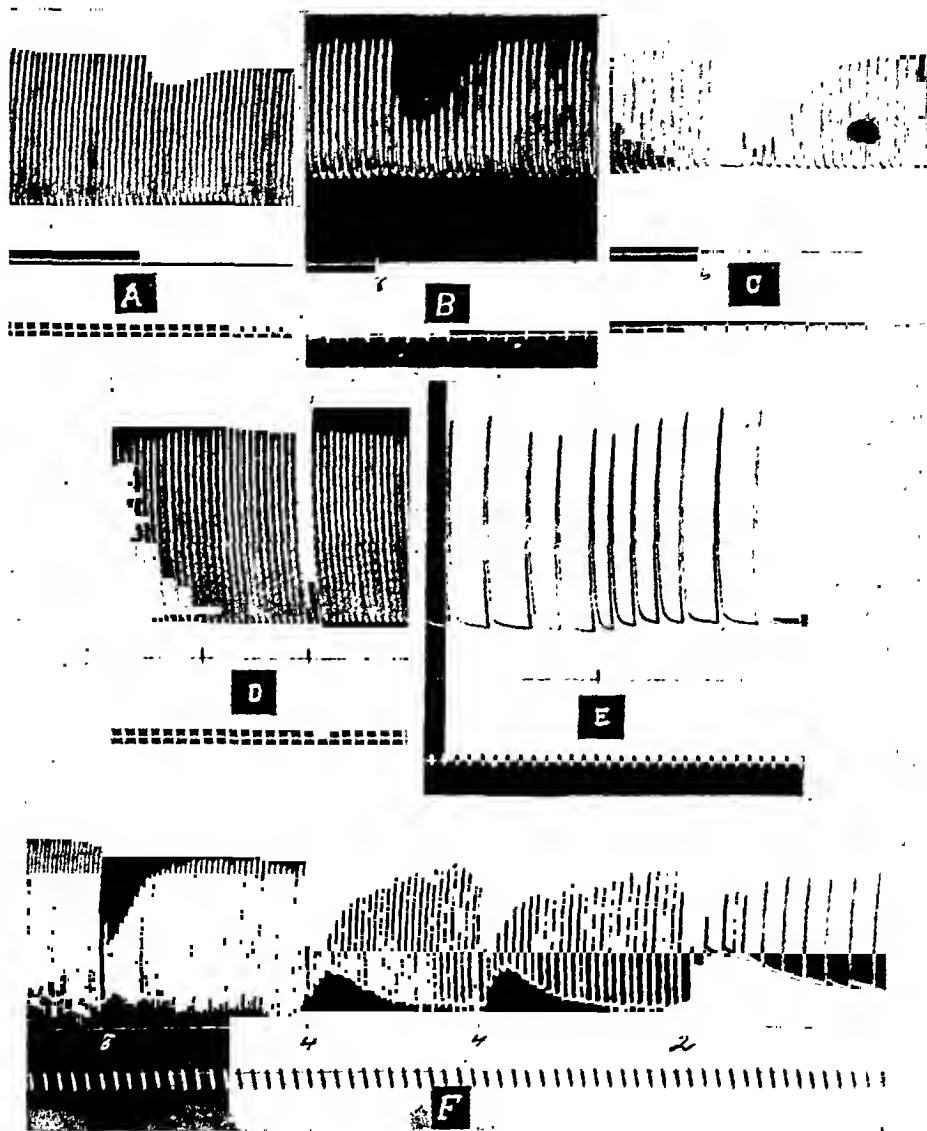


Fig. 1. A, B and C. Inhibition following single break shocks. Harvard inductorium primary=6 v. secondary coil at 9 cm., 8 cm., and 6 cm. Time = 5 sec. Signal indicates stimulation.

D and E. Single break shocks after atropinization. Primary 6 v.; secondary 2 cm. E. shows quickening after stimulation. Time = 5 sec.

F. Preparation previously immersed in 1-6000 solution of eserine chloride. Progressive diminution in frequency and amplitude after each single shock. Time = 5 sec.

result has been plotted as percentage depression against time. The amount of inhibition and its duration varied with the strength of stimulus and condition of the preparation but under similar conditions the curves are remarkably uniform.

Cooling the preparation lengthened the time values of the curve and raised the threshold slightly.

A supernormal phase, a conspicuous after-effect following inhibition produced by repetitive stimulation (6) did not appear after single shocks. It seems probable that the repetitive stimuli which cause inhibition simultaneously liberate an excitatory substance, e.g., adrenaline or sympathin, at sympathetic endings. This persists longer than the inhibitory substance and its effects appear after the inhibition has disappeared. The supernormal phase was more pronounced when inhibitory excitation was prolonged. Apparently a single shock did not cause the release of enough excitatory substance to outlast the depression, therefore no supernormal phase appeared.

To the extent that the above described inhibition is humoral, acetylcholine being liberated at vagus terminals, atropine should abolish it and eserine should potentiate or increase it. Proceeding to test these possibilities, atrial preparations were atropinized either by injecting the drug shortly before removal of the atria or by immersing the preparation in atropine-Ringer solution. As a result the inhibiting effect of single shocks was prevented or removed (fig. 1, D

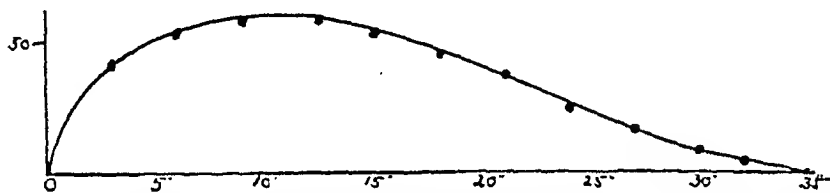


Fig. 2. Inhibitory effect of a single break shock plotted as per cent depression against time. Secondary = 8 cm.

and E). Other than the compensatory pause which follows a premature beat, there was no slowing of the rhythm and no diminution of amplitude after induction shocks well above the normal threshold for such effects. In some instances a brief quickening was observed (fig. 1, E). This may have been due to the excitation of sympathetic endings by the stimulus.

According to Sollmann (7), eserine stimulates the frog's heart muscle and antagonizes both the vagus and the effect of depressants. There was evidence of a similar effect on the turtle's atria since preparations beat more rapidly and vigorously when immersed in strong solutions, e.g., 1-1000 eserine chloride in Ringer. Under such conditions the threshold for inhibition was raised. However the main action of eserine was to prevent or delay recovery from inhibition. This action was best demonstrated by applying single inhibiting shocks at one or two minute intervals. Under these conditions there was incomplete recovery after each stimulus and the frequency and amplitude of beat were depressed progressively until after a succession of strong shocks the beat was completely arrested. Figure 1, F, illustrates these effects. The cumulative effect and arrest under eserine could be removed and the beat restored by atropine.

Incidentally it was noted that eserinizied preparations were very sensitive to

inhibition by mechanical as well as electrical stimuli. When animals were eserinizied previously, the procedure of removing and suspending the preparation was usually sufficient to arrest the beat completely. This mechanically induced arrest could be removed and the beat restored by atropine.

DISCUSSION. An electric shock sent through an atrial preparation as described comes into excitation relations not only with the cardiac muscle itself but also with ganglion cells and with the fibers and terminals of sympathetic and parasympathetic nerves. It is the algebraic sum of these excitatory and inhibitory effects which is recorded by the muscle. The experiments reported here show that when the stimulus reaches a sufficient intensity, the inhibiting effect predominates, the beat is slowed and the amplitude diminished. This depression could originate through several possible mechanisms. Membrane potentials or membrane permeability could be altered. There could be actual change in the structural arrangement or chemistry of the cytoplasm. Finally acetylcholine could be released at vagal terminals and this account for the observed effect. The following facts strongly support the latter conclusion. Atropinization prevents or eliminates the inhibition. The inhibition of both amplitude and frequency of contraction is progressively cumulative after eserine. The threshold is raised and the time relations prolonged by cooling. The preparation recovers its previous frequency and force within a minute after the reception of the inhibitory stimulus. It is significant that when the inotropic effects of single electrical shocks are expressed as a time-depression curve, its form is practically identical with those which Gilson (8) plotted from single vagal volleys. Gilson found such curves to show considerable agreement with curves constructed from an equation expressing the diffusion and destruction of an assumed chemical substance.

On the basis of above evidence it seems possible to conclude that a single shock of sufficient strength releases a humoral substance in quantity sufficient to overcome excitatory effects and depress both amplitude and frequency. A short time is required for the humor to reach maximum concentration at effective points and it is more slowly destroyed. Experiments employing atropine and eserine as well as the close correspondence to effects of single vagal volleys give strong support to the conclusion that acetylcholine released at vagus terminals is the inhibiting agent. The finding that no inhibition can be demonstrated when the ventricle or ventricular strips are similarly stimulated may be cited as indirect evidence for the above conclusion since Garrey and Chastain (9) showed that the ventricle which receives no vagus fibers is not affected by acetylcholine.

SUMMARY

Inhibition of the turtle's atria may be produced by the direct application of single brief electrical shocks provided they are of sufficient intensity. The threshold for such effects is comparatively high, lying much above the intensity necessary for excitation. The difference between the relative effectiveness of repetitive and single shocks lies mainly in the high threshold for the latter.

It requires a short time for the depression to reach its maximum. It then slowly disappears.

The prevention of the inhibition by atropine and its prolongation by eserine indicate that acetylcholine is liberated at vagal terminals in sufficient quantity to inhibit.

The similarity of time-depression curves to curves constructed from effects of single vagal volleys is noted.

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THE ADRENALS AND HYPOPHYSIS IN THE CARBOHYDRATE METABOLISM OF THE EVISCERATED RAT¹

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Hypophysectomized rats and rabbits from which the abdominal viscera have been removed require the administration of much more glucose to maintain their blood sugar levels than do normal eviscerated animals. The hypophysectomized eviscerated rat also exhibits a loss of muscle glycogen which control animals do not show (1, 2). These observations substantiate those made previously on intact hypophysectomized animals and on animals given pituitary extracts—observations which suggest that in the absence of the anterior pituitary there is abnormally great utilization of carbohydrate in the peripheral tissues in circumstances when carbohydrate oxidation is ordinarily not rapid (3, 4). The hypophysectomized animal also suffers from some degree of adrenal cortical insufficiency. Since the adrenal cortical hormone is known to affect gluconeogenesis, it is now generally considered that the changes in hepatic metabolism of carbohydrate observed in certain circumstances after hypophysectomy are due to this deficiency. There is some evidence which has been considered to indicate that the administration of cortical hormones, besides increasing gluconeogenesis, also decreases carbohydrate utilization. Whether this effect, if real, is hepatic or peripheral in origin is not known, nor has it been decided whether adrenal insufficiency, like hypophyseal deficiency, leads to increased peripheral oxidation of carbohydrate. Consequently, whether any of the effects of the absence of the pituitary on peripheral carbohydrate metabolism are mediated through the adrenal cortex is likewise unknown. The experiments reported here, in continuation of previous work on eviscerated rats, concern, first, the confirmation of peripheral effects of hypophysectomy by the reversal of these changes following the administration of anterior pituitary extracts, and secondly, attempts to answer in part the questions just posed by a comparison of the effects of adrenalectomy and of hypophysectomy, and of pituitary preparations and cortical hormones on peripheral carbohydrate metabolism.

METHODS. All chemical, operative and infusion techniques used in these experiments were the same as those previously employed (1). The animals were, as before, young male rats of the Sprague-Dawley strain, unfasted.

The anterior pituitary extract (A.P.E.) used was a 2 per cent saline extract of frozen beef anterior lobes, 1 ml. containing about 10 mgm. of protein and representing 0.2 gram of gland. A total dose of 1 ml. per 100 grams body weight was administered intra-peritoneally at intervals during 4 hours preceding an experi-

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ment; in some cases, part of the dose was given subcutaneously after evisceration. Administration of the extract over longer periods of time (up to 24 hrs.) did not seem to be more effective in these experiments than administration as just described.

The adrenal cortical extract was Upjohn's Adrenal Cortical Extract, 1 ml. representing 40 grams of gland and containing 50 dog units or 2.5 rat units as assayed by the manufacturer. Alcohol used as a preservative was removed by vacuum distillation before the extract was injected. Total doses of 0.1 to 0.6 ml. per 100 grams body weight were given in 3 equal parts: intraperitoneally $\frac{1}{2}$ hour before experiment, and subcutaneously immediately after evisceration and again 1 hour later. Desoxycorticosterone acetate pellets weighing about 100 mgm. were implanted subcutaneously, 1 in each animal, from 3 to 10 days before experiment. The pellets were weighed before implantation and after removal from the animal; the loss of weight of the pellets averaged 0.4 mgm. per day.

As before, the infusion experiments were conducted over a 2 hour period, 0.2 ml. samples of tail blood being taken for sugar determinations at half-hour intervals. After the cessation of a glucose infusion, a blood sample was taken 1 hour later or on the death of the animal if that occurred earlier. When the eviscerated animals were not given glucose, blood and muscle (gastrocnemius) samples were taken immediately after evisceration and again 1 hour later.

All of the control or "normal" animals were adrenal-demedullated two weeks or more prior to experiment. The hypophysectomized rats were adrenal demedullated two weeks or more before operation and were used for experiment two weeks or more after hypophysectomy. The adrenalectomized rats were used from 4 to 14 days after operation, maintained meanwhile by the addition of sodium salts to the drinking water (0.7 per cent sodium chloride, 0.3 per cent sodium bicarbonate). No salt was given to animals carrying desoxycorticosterone pellets. All adrenalectomized rats, supported either with salt or with desoxycorticosterone, grew well during this time. Desoxycorticosterone treatment did not affect the weight of the hypophysectomized rats.

RESULTS. The results of the infusion experiments are presented in figure 1. In each experiment, the differences from the initial blood sugar levels were taken, and then for each group of experiments, the mean and standard error of the mean of these differences were calculated for each time interval. The mean differences were then plotted as average curves for each group of experiments. The standard errors, omitted from the figure for the sake of clarity, were not large, varying from 1.5 to 6.4 and averaging 3.4 mgm. per cent. Where two means have been compared, the significance of the difference between the means has been determined by Fisher's "t" test for small samples.

The initial blood sugar levels lay usually between 70 and 90 mgm. per cent. The several series of experiments did not differ significantly with regard to their mean initial blood sugar levels.

A.P.E. in normal eviscerated rats. The normal (adrenal-demedullated) eviscerated rat was previously shown (1) to require for the maintenance of a normal blood sugar level the administration of 13 to 14 mgm. of glucose per 100 grams

(initial body weight) per hour; the average curve is shown as group B, figure 1. In the present series of experiments it was first determined that after the ad-

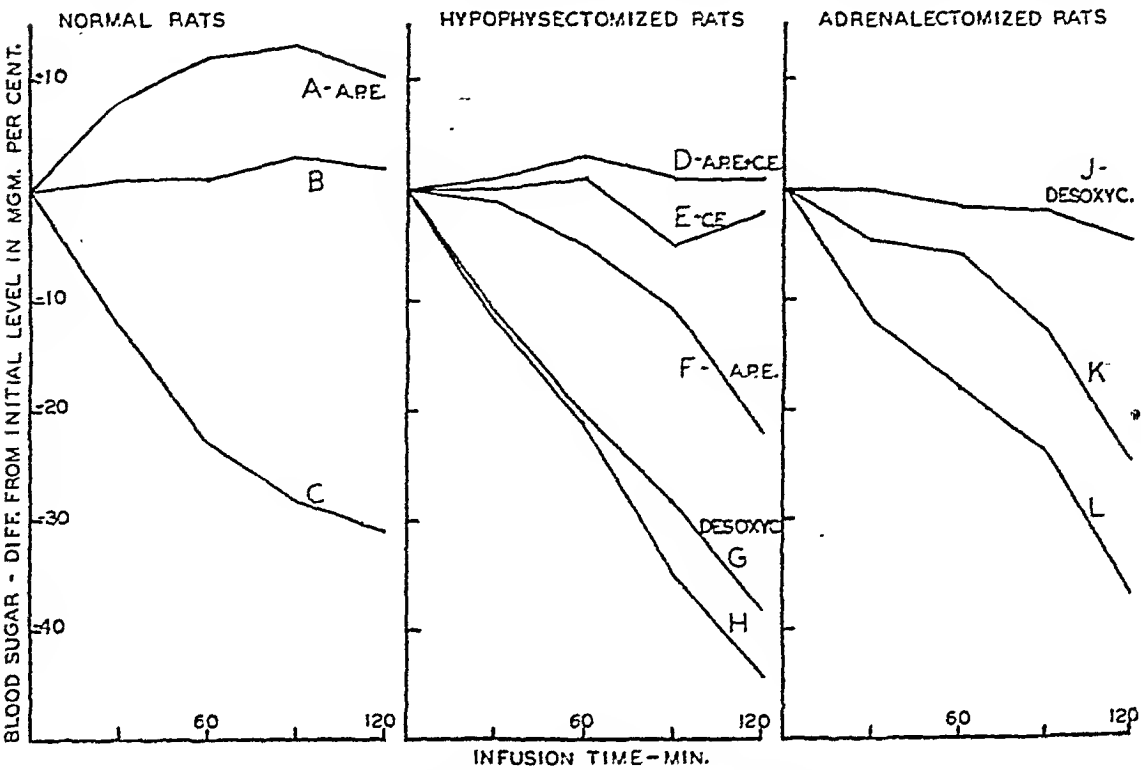


Fig. 1. Maintenance of the blood sugar by intravenous glucose infusion in eviscerated rats.

SERIES	NO. OF ANI-MAIS	TREATMENT*	GLUCOSE INTUSED** MG. PER 100 GRAMS PER HOUR
A. Normal (adrenodemedullated) rats.....	9	Ant. pit. ext.	8.3
B. Normal (adrenodemedullated) rats.....	7		13.7
C. Normal (adrenodemedullated) rats.....	9		9.2
D. Hypophysectomized (adrenodemedullated) rats.....	4	Ant. pit. ext., cortical ext.	13.2
E. Hypophysectomized (adrenodemedullated) rats.....	7	Cortical ext.	13.3
F. Hypophysectomized (adrenodemedullated) rats.....	8	Ant. pit. ext.	14.0
G. Hypophysectomized (adrenodemedullated) rats.....	7	Desoxycorticosterone	13.6
H. Hypophysectomized (adrenodemedullated) rats.....	10		14.0
J. Adrenalectomized rats.....	6	Desoxycorticosterone	14.0
K. Adrenalectomized rats.....	7	Salt	20.0
L. Adrenalectomized rats.....	3	Salt	14.0

* See text for details of treatment.
** Average glucose infusion rate.

ministration of A.P.E. as described above, the glucose requirement was not more than 8 mgm. per 100 grams per hour. Simultaneously, a similar series of experiments was performed in rats not given A.P.E. but infused with similar amounts of glucose (8 to 10 mgm. per 100 grams per hr.). In these animals the blood sugar always fell progressively. The mean curves of the two groups of experiments, showing the differences from initial sugar levels, are presented in figure 1, groups A and C. The differences between the curves for the treated and untreated rats are highly significant at all points.

In comparison with the rate of utilization of carbohydrate by normal eviscerated rats, the glucose requirement has been reduced at least 40 per cent by the A.P.E. treatment. As far as these data show this reduction may be due to a diminution either in the deposition of glycogen or in the oxidation of carbohydrate by the peripheral tissues. However, since in control eviscerate rats, either maintained with glucose or not, there is no change in muscle glycogen or in blood lactate (1), and since in the intact rat A.P.E. causes increased deposition rather than disappearance of muscle glycogen (4), it seems probable that here the effect of A.P.E. has been to decrease peripheral oxidation of carbohydrate.

A.P.E. in hypophysectomized rats. Previously it was reported that A.P.E. treatment of hypophysectomized rats reduced the rate of fall of blood sugar after evisceration and prevented the disappearance of muscle glycogen which otherwise occurred (1). In the present experiments the amount of glucose needed by the normal rat was given to the A.P.E. treated hypophysectomized rat and the resulting blood sugar curves were compared with the curves obtained when similar amounts of sugar were given to untreated hypophysectomized rats (fig. 1, curves *F* and *H*). As expected, the glucose requirement was reduced by the administration of A.P.E., although the restoration toward normal was not quite complete under these circumstances. During the first hour, the points on this curve, (*F*), do not differ significantly from zero, indicating a normal utilization rate. Later the blood sugar level fell noticeably, but at all points it remained significantly above the curve for untreated animals. Following the infusion, the rate of fall of the blood sugar of the treated animals was normal (table 1).

Adrenal cortical hormones in hypophysectomized rats. The administration of whole adrenal cortical extract to eviscerated hypophysectomized rats allowed the blood sugar to be maintained by the infusion of the same amount of glucose as is required by normal eviscerated rats (fig. 1, group *E*). The amounts of extract used, 0.2 to 0.6 ml. per 100 grams body weight, were all about equally effective; smaller doses (0.1 ml. per 100 grams) were only partially so. These amounts of extract sufficed only when they were given in divided doses through the experimental period, a single injection prior to evisceration having only a temporary effect. The effects of A.P.E. and cortical extract were not noticeably synergistic. As shown in figure 1, treatment with both A.P.E. and cortical extract in the same amounts as were used separately was little more effective than the cortin alone. In other experiments (not shown), A.P.E. was found to be no more effective in the presence of smaller amounts of cortical extract than when given by itself.

In hypophysectomized rats which were eviscerated but not given glucose, whole adrenal cortical extract increased the survival time and decreased the rate

of fall of the blood sugar to normal, but only partially prevented the loss of muscle glycogen (table 2).

The implantation of pellets of desoxycorticosterone acetate into hypophysectomized rats did not affect their glucose requirement, the blood sugar curves from untreated and desoxycorticosterone treated rats not differing significantly.

TABLE 1

The rate of fall of the blood sugar in eviscerated rats after the cessation of glucose infusions

	NO. OF EXPERIMENTS	RATE OF FALL OF THE BLOOD SUGAR
		<i>mgm. per cent per hour</i>
1. Normal rats.....	12	33 ± 4.6*
2. Normal rats treated with anterior pituitary extract.....	9	24 ± 5.9
3. Hypophysectomized rats.....	12	78 ± 4.5
4. Hypophysectomized rats with desoxycorticosterone pellets..	6	60 ± 7.2
5. Hypophysectomized rats treated with adrenal cortical extract (0.2-0.6 ml. per 100 grams).....	8	31 ± 2.8
6. Hypophysectomized rats treated with anterior pituitary extract (1 ml. per 100 grams).....	6	31 ± 3.9
7. Hypophysectomized rats treated with both adrenal and pituitary extracts (same amounts as above).....	6	37 ± 2.5
8. Adrenalectomized rats, salt treated.....	7	68 ± 6.1
9. Adrenalectomized rats, with desoxycorticosterone pellets.....	8	40 ± 3.0

* Standard error.

TABLE 2

Carbohydrate levels in eviscerated rats not given glucose

	NO. OF RATS	SURVIVAL TIME	BLOOD SUGAR		MUSCLE GLYCOGEN	
			Initial	Rate of fall	Initial	Rate of change
		<i>minutes</i>	<i>mgm. %</i>	<i>mgm. % per hour</i>	<i>mgm. %</i>	<i>mgm. % per hour</i>
1. Control demedullated rats.....	12	107 ± 10	82	37 ± 3	609	+2 ± 11
2. Adrenalectomized rats given salt.....	8	83 ± 3	88	60 ± 6	516	-2 ± 11
3. Hypophysectomized rats, untreated.....	13	53 ± 4	76	72 ± 6	529	-108 ± 16
4. Hypophysectomized rats given cortical extract*.....	10	96 ± 10	77	41 ± 4	573	-42 ± 13

* Upjohn's Adrenal Cortical Extract, 0.5 ml. per 100 grams body weight 2 hours before experiment.

The rate of fall of the blood sugar after the cessation of glucose infusion was in the treated animals slightly lower than in untreated rats (the difference being barely significant), but it was still far above the normal rate.

The eviscerated adrenalectomized rat. The adrenalectomized rat maintained in good condition by the administration of salt behaves after evisceration somewhat

like the hypophysectomized rat. The blood sugar falls more rapidly than normal when no glucose is given, and the glucose requirement for maintenance of the blood sugar is increased (tables 1 and 2, fig. 1, groups *K* and *L*). The rate at which glucose must be given to the salt treated adrenalectomized animal is variable and so is difficult to determine precisely, but it appears to be above 20 mgm. per 100 grams per hour.

The adrenalectomized rat also differs from the hypophysectomized rat in important particulars. The muscle glycogen does not fall after evisceration as it does in the hypophysectomized rat, and the survival time after evisceration is longer (table 2). These facts probably indicate that the rate of carbohydrate utilization is less in salt treated adrenalectomized rats than in hypophysectomized rats. More striking still is the difference in the response of the two types of animals to implantation of desoxycorticosterone acetate pellets. Whereas the hypophysectomized eviscerated rat was affected only slightly if at all, the adrenalectomized animal maintained with desoxycorticosterone (without salt) required only normal amounts of glucose and the rate of fall of the blood sugar after cessation of glucose infusion was within normal limits (fig. 1, curve *J* and table 1).

DISCUSSION. It appears established that hypophysectomy leads to an increase in the glucose requirement of the peripheral tissues and that A.P.E. treatment reverses these effects both in normal and in hypophysectomized animals. But since salt-treated adrenalectomized rats also show some increase in their peripheral utilization of carbohydrate, may this effect of hypophysectomy be due in part to adrenal cortical atrophy? The fact that maintenance doses of desoxycorticosterone restored the peripheral carbohydrate utilization rate of adrenalectomized rats but did not affect that of hypophysectomized rats argues against this conception. If such doses of desoxycorticosterone do not affect carbohydrate metabolism, as many believe, the adrenalectomized rat *when in the best condition* might be said to have a normal rate of utilization of carbohydrate, and adrenal insufficiency could not then be an important factor in producing the increased glucose requirement after hypophysectomy.

The view that adrenalectomy does not specifically increase carbohydrate utilization is also supported by the fact that the R.Q. of adrenalectomized animals is generally normal (5, 6, 7, 8). Normal or nearly normal deposition of glycogen or recovery of absorbed carbohydrate in adrenalectomized rats fed glucose has been reported several times (5, 9, 10). Two reports, those of Lewis et al. (11) and of Evans (12), are in conflict with this view, but, on examination, the data they present do not appear to support the conclusion that disappearance of administered carbohydrate is notably accelerated after adrenalectomy.

In view of the small effects of adrenalectomy on carbohydrate utilization, the similar results of the administration of adrenal cortical hormones and of anterior pituitary extracts in the present experiments are somewhat enigmatic. Evidence has been considered elsewhere (13, 14) to indicate that the anterior pituitary can affect carbohydrate metabolism independently of adrenal activity and that the principal effects of cortical hormones on metabolism are referable to gluconeogenesis; but it may be that cortical hormones given in sufficient quantity

can also affect directly or indirectly the rate of carbohydrate utilization. However, whether anterior pituitary and adrenal extracts act in an identical manner is not shown by these experiments, for the metabolism of peripheral tissues as well as that of the intact animal may be affected by administered hormones at more than one point. It seems probable that the anterior pituitary acts directly upon carbohydrate metabolism in some manner, for effects on muscle glycogen are peculiar to A.P.E.; but cortical extract may, for instance, affect the availability of fat for direct oxidation, or it might produce its apparent effect by increasing gluconeogenesis in kidney tissue (15). Evidence obtained from the action of cortical hormones in intact animals is not helpful in deciding this point. In most experiments, on diabetic, phlorizinized, or fasted rats, cortical hormones have appeared only to increase gluconeogenesis (14, 16). Only in rats fed large amounts of carbohydrate have adrenal steroids depressed the R.Q. (5, 14), or, in one instance, caused excessive glucose excretion (17); and in these cases there may be some doubt that direct repression of carbohydrate oxidation by the hormones occurred. The low R.Q.'s, presumed to indicate a reduction in carbohydrate oxidation, were accompanied by liver glycogen but not by peripheral carbohydrate storage; so they may then as well have been the secondary results of abnormal retention of carbohydrate in the liver, or even perhaps have been due to a reduction in fat formation. The high rate of glucose excretion could have been due to hyperglycemia, produced when massive carbohydrate feeding was superimposed on prevailing high carbohydrate levels due to gluconeogenesis. Evidently it is still necessary to reserve judgment upon the nature of peripheral effects of active cortical hormones on metabolism.

A question of considerable importance brought up by the present experiments concerns the rôle of desoxycorticosterone in carbohydrate metabolism. The consensus is that desoxycorticosterone in small or moderate doses does not affect carbohydrate metabolism in any demonstrable fashion, but some effects of larger amounts of desoxycorticosterone on gluconeogenesis and on the blood sugar level have been reported (16, 18). It is possible therefore that the eviscerated rat is a preparation particularly sensitive to the presence or absence of adrenal hormones, and that the increased rate of utilization of carbohydrate observed in salt-treated adrenalectomized rats and the restoration to normal by desoxycorticosterone are real effects. On the other hand, another explanation for the present effects of desoxycorticosterone is possible. It has been reported several times in the past that when hepatectomized or eviscerated animals are approaching shock states ("in a declining condition") the glucose requirement is elevated (19); and in this work on eviscerated rats a similar phenomenon was also noted. Some experiments have been performed (to be reported elsewhere) showing clearly that after hemorrhage the glucose utilization rate of eviscerated rats is much accelerated. It is well known that adrenalectomized animals are particularly susceptible to shock-inducing procedures and that they may often be protected by the administration of desoxycorticosterone. These facts suggest that the increased glucose requirement of salt-treated adrenalectomized eviscerated rats may be due not specifically to lack of an adrenal hormone directly affecting car-

bohydrate oxidation by the tissues, but to the early development of a shock state after evisceration. The desoxycorticosterone may then produce its apparent effect on carbohydrate utilization by action on the peripheral vascular circulation. If the defect in carbohydrate metabolism in hypophysectomized animals is referable to lack of a pituitary rather than of an adrenal factor, as previously considered, the failure of desoxycorticosterone in these animals is to be expected.

However, it is probably not possible to divorce desoxycorticosterone entirely from any participation in metabolism; for the susceptibility to shock of adrenalectomized animals and their protection by adrenal hormones may itself be due in part to the participation of adrenal hormones in metabolic systems affected in the syndrome known as shock.

SUMMARY

1. The glucose requirement for maintenance of the blood sugar in normal eviscerated rats was reduced by about 40 per cent by previous treatment of the animals with anterior pituitary extract.

2. The glucose requirement of eviscerated hypophysectomized rats, which is greater than normal, was reduced by anterior pituitary and by adrenal cortical hormone treatment. The rate of fall of the blood sugar when no glucose was given was reduced to normal by both types of treatment. The loss of muscle glycogen, which occurs in untreated hypophysectomized eviscerated rats and which is prevented by A.P.E. treatment, was only partially affected by adrenal cortical extract. Desoxycorticosterone acetate did not alter the glucose requirements of hypophysectomized eviscerated rats.

3. Adrenalectomized rats sustained by the administration of salt required more glucose than normal rats for maintenance of the blood sugar after evisceration. The blood sugar fell more rapidly than normal in these rats when no glucose was given, but the muscle glycogen did not change during the period studied. Adrenalectomized rats maintained with desoxycorticosterone acetate (subcutaneous pellets) required only normal amounts of glucose after evisceration.

4. The significance of these observations has been discussed. Reasons were advanced for considering that the increased peripheral requirement for carbohydrate of hypophysectomized rats is probably not to any great extent the result of diminished adrenal function and that the pituitary and adrenal hormones may affect carbohydrate metabolism by different means.

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OBSERVATIONS ON INJURY AND REPAIR OF PERIPHERAL NERVES

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The desirability of more complete information on the many physiologic processes involved in the repair of injured nerves is universally recognized. It is only by acquiring such knowledge that rational methods of treatment of injured nerves may be evolved. Through the application of certain technics, we have observed *in vivo* the repair of the posterior auricular nerve of the rabbit after the nerve had been injured in a variety of ways. Although our study is far from complete, it has been of sufficient promise to warrant a report of our findings to date.

Recently one of us (Rezende, 6) reported the results of a series of experiments on grafting and suturing divided peripheral nerves. In that paper considerable pertinent literature was presented. Furthermore a very inclusive bibliography has been published recently by Young (8) in his able review of the subject of injury and repair of peripheral nerves. In addition, two important papers have appeared recently, one by Gutmann and Sanders (4) and the other by Weiss and Taylor (7). Consequently only the literature that bears an intimate relation to the present study will be referred to subsequently in this communication.

METHODS. A transparent chamber applied to the ears of rabbits by means of the technic of the Clarks and their co-workers (2) has been used in this laboratory for several years. Clark, Clark and Williams (2) described the ingrowth of nerves that accompanied the developing blood vessels in the transparent chamber inserted in the ears of rabbits. Because of the fact that living nerves are translucent, they are indistinguishable from other tissues when seen with the microscope. Clark, Clark and Williams stained the newly formed nerves by injections of methylene blue in concentrations of 1:50, 1:200, and 1:400 in physiologic saline solution. The solution was injected into an artery or a vein in sufficient amount to distend the vessels of the chamber with the dye. They occluded the circulation for one and a half to three minutes while the dye was kept in the vessels. Seven to ten minutes later the nerves were stained an intense blue which persisted for thirty-seven to forty-three minutes.

In the experiments reported here the method of Clark, Clark and Williams for staining the nerves was used. Effective concentrations of the dye that appeared to be tolerated without serious untoward effects were 2.5 to 5 per cent solutions of methylene blue in physiologic saline solution. Concentrations of 15 to 20 per cent caused considerable edema of the whole ear as the Clarks and Williams found with high concentrations of the dye.

Besides observing the repair of the nerve following injury, we determined loss

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of function and its recovery by stimulating the ear by appropriate means and observing the response of the animal.

The transparent chamber. For the present study a transparent chamber similar to that used by the Clarks and their co-workers for the study of preformed or pre-existing blood vessels was employed. The chamber we used was made from lucite. The two disks which made up the upper and lower portions of the chamber were cut on a turning lathe from a cylinder of lucite about 25 mm. in diameter after the method of Ebert, Florey and Pullinger (3). The lower disk when polished measured from 1.75 to 2 mm. in thickness while the upper disk was made about 1 mm. thick. Three holes about 1 mm. in diameter were drilled through the two disks in the form of an equilateral triangle to receive the bolts for holding together the upper and lower disks of the chamber. From the upper disk a smaller disk about 15 mm. in diameter was removed and a layer of mica about 0.07 mm. thick was glued to the lower surface with a solution of copal in acetone and Venice turpentine. The mica and lucite were held in a clamp for about twenty-four hours, after which the bolts were placed in position, the nuts applied and the chamber thus assembled was submerged in 10 per cent solution of formalin or 1:1,000 solution of merthiolate for 60 to 120 minutes. After the chamber had been rinsed in physiologic saline solution, it was inserted into the ear of the rabbit.

The insertion of the chamber. The operations were done while the animals were under pentobarbital sodium anesthesia (25-35 mgm. for each kilogram of body weight given intravenously). The ear was clipped, washed with soap and water and placed in 1:500 solution of metaphen or 1:1,000 solution of merthiolate for fifteen minutes. The antiseptic was placed in a 50 cc. centrifuge tube in which the ear was submerged. Precautions to ensure sterility were observed throughout the operation.

The procedures for insertion of the chamber were as follows: 1. The upper portion or disk of the window was placed so that the medial artery passed near the middle of the disk. 2. The ear was spread on a large cork and when the disk was placed satisfactorily, sewing needles were thrust through the ear into the cork to mark the position of the bolts which were to be inserted later. 3. The upper disk was then removed and with a scalpel having a narrow, thin blade the skin was cut through and separated from the blood vessels and cartilage for a few millimeters. After this a blunt probe was inserted and the skin was elevated from the cartilage about 5 to 8 mm. beyond the area to be covered with the upper disk of the chamber. The skin on the ventral surface was not disturbed. 4. From the center the skin was incised radially to each of the sewing needles but not beyond it. 5. The sewing needle was passed into the lumen of the hypodermic needles and the latter was then thrust through the ear; the sewing needles were removed, after which the bolts were inserted into the hypodermic needle. The hypodermic needle was then removed and the bolt left in place through the ear. 6. After the three bolts were placed the lower disk was put in position and the nuts were applied. 7. If the nerve was to be sectioned or a graft placed, it was done at this point. 8. The upper disk was then put in position, the bolts were

placed and the nuts applied. 9. The skin was then brought over the periphery of the upper disk and trimmed just sufficiently to expose the central disk of mica. 10. The nuts were then tightened to the desired degree, which completed the procedure. The entire operation can be done in twenty minutes.

Observations of the tissues in the chamber were made with the aid of a dissecting and a compound microscope.

DESCRIPTION OF EXPERIMENTS AND RESULTS. One of the most striking developments in the experiments thus far has been the response of the circulation of the nerve to injury. In a recent review Adams (1) emphasized the fact that normal peripheral nerves as well as the central nervous system are highly vascular. Our observations on living functioning nerves strongly support this thesis. Regardless of the nature of the injury to which the auricular nerves were subjected, the first occurrence thereafter was the extensive development of a complex network of blood vessels which formed in the crushed region of the nerve, or which spanned the gap between the proximal and distal stumps when the nerve was sectioned or when part of the nerve was resected. Similarly when a graft was placed in the course of the nerve, it was first completely vascularized whether it was an autograft or a homograft. This was true whether it was a graft made from fresh or from preserved nervous tissue. The graft, particularly when made from preserved material, could be distinguished for several days as an opaque area of diminishing size in the course of the nerve. Vascularization occurred much more rapidly (seven to ten days) in the fresh than in the preserved material whether an autograft or a homograft was used. In the case of homografts made from preserved nerves, what appeared to be complete vascularization required fourteen to twenty-one days. In all experiments the initial vascularization appeared to be in excess of the ultimate needs of the nerve since the complex network of vessels gradually became reduced until the vessels of the region of injury or of the graft could be distinguished from those in the proximal and distal portions of the nerve only with considerable difficulty. That is, the vascular architecture finally took on the appearance of that of the normal nerve.

Following the vascularization of the injured region or of the graft, ingrowth of nervous tissue occurred after varying lengths of time. The limited number of experiments done in any of the following series of experiments makes it advisable to consider the statement of the time required to produce a given result as tentative since a much larger series of experiments must be done before an adequate idea of the range of time required for a given result can be had.

Series 1 (4 expts.). In a series of control experiments 2.5 per cent solution of methylene blue was injected through the marginal vein of the ear, the injected dye being held in place by preventing circulation of blood as described by the Clarks and Williams. Fifteen minutes later the vessels and nerves were denuded of skin, care being taken to cause as little injury to the underlying blood vessels and nerves as possible. Under optimal conditions the skin may be removed without gross hemorrhage being produced. An examination of the tissues thus exposed revealed deep blue bands of various diameters passing along the medial auricular artery with secondary bands passing laterally into the adjacent tissue. The

former were components of the posterior auricular nerve and the latter were its branches. The deep blue staining bands have been interpreted as the axis-cylinders of the posterior auricular nerve and its branches (fig. 1).

Series 2 (18 expts.). In this series of control experiments the transparent chambers were applied to the ears with the least possible disturbance of the blood vessels and nerves. When complete recovery had occurred, which required ten to fourteen days, the tissues of the chamber were stained for three to five minutes with appropriate concentrations of the dye. It was demonstrated that the portion of the nerves that had been placed in the chambers and allowed to remain there indefinitely stained as did the nerves in the ears of the rabbits in series 1 (fig. 2).

Series 3a (6 expts.). From the observations just described, it was evident that the presence of the chamber did not alter the staining character of the nerves. In the series now under discussion the nerve was exposed, separated from the medial artery for a short distance and then crushed between forceps for a distance of about 5 mm., after which the chamber was applied. The sequence of events was then followed until recovery or until the preparation failed for some reason. Only two of the preparations in this series were successful, since in the other preparations the tissues of the chambers became infected after various periods following the operation. Within fourteen days following the operation the vessels of the nerves that had been crushed appeared to have recovered fully from their injury. The vascular architecture looked quite as it did in an uncrushed nerve (fig. 3a and b). In one of the animals the crushed nerve had apparently fully recovered its function seventy-two days after injury, since the animal responded to stimulation of the ear in an area supplied by the posterior auricular nerve, which it did not do just after the operation. Staining with methylene blue showed that axis-cylinders had grown across the injured region into the distal uninjured portion of the nerve.

Series 3b (4 expts.) In these experiments the transparent chambers were applied as described in series 2. After complete recovery from the operation in certain of the animals the tissues of the chamber were stained with methylene blue to determine the condition of the nerve. If the preparation was in every way satisfactory at a later date, the nerve was crushed near the base of the ear as in series 3a. The operation was done quickly and easily while the area was under infiltration anesthesia with procaine hydrochloride. Twenty-four to forty-eight hours after the operation the ear was stimulated to determine the presence or absence of function of the nerve. In every instance the injury of the nerve was sufficient to destroy its function. Five days after the nerves were crushed, they failed to stain as they did before being crushed. There were only one or two faintly staining bands, a fact which indicated that the axis-cylinders had almost completely degenerated in each instance. Experiments are now in progress to determine the time necessary for the axis-cylinders to regenerate from a point proximal to the injury through the transparent chamber, which is a distance of 5 to 10 cm.

Series 4. Resection. In one experiment a piece of the posterior auricular nerve about 3 or 4 mm. long was resected. In addition there was enough retraction of

the nerve to produce a gap about 5 mm. long. The space between the proximal and distal stumps was bridged within five days by a rich supply of blood vessels

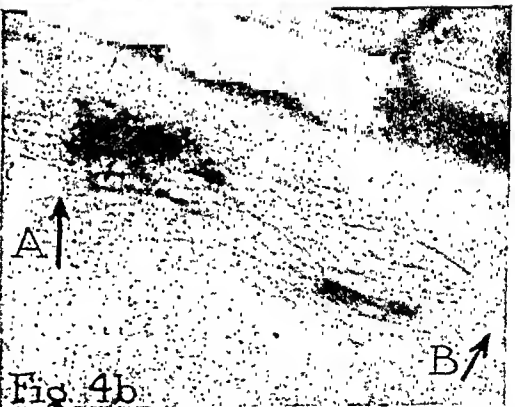
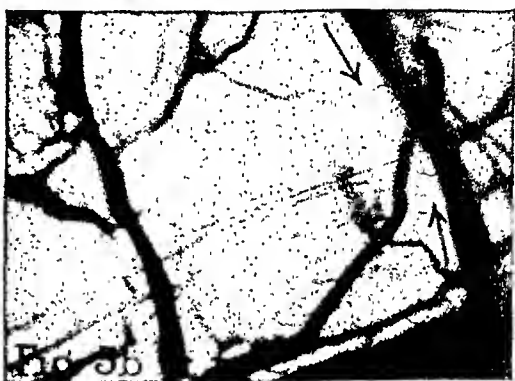
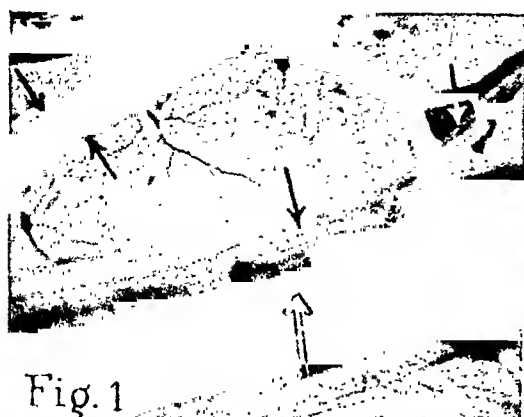


Fig. 1. Denuded central artery and posterior auricular nerve with two of its branches as seen in the ear of the rabbit after staining with 2.5 per cent solution of methylene blue ($\times 5$).

Fig. 2. Tissues in transparent chamber inserted in the ear of a rabbit. Picture taken after staining tissues with methylene blue. Nerve indicated by deeply stained bands ($\times 5$).

Fig. 3a. Transparent chamber showing effect of crushing posterior auricular nerve ($\times 5$). b. Same fourteen days later when circulatory repair was largely completed.

Fig. 4a. Tissues in transparent chamber, unstained; blood vessels and posterior auricular nerve from which a piece (A to B) had been resected sixty-four days previously ($\times 5$). b. Same after staining with methylene blue. The bundles of nerve fibers can be seen passing from the tip of a neuroma (A) to the distal stump (B) ($\times 8$).

whose development preceded the ingrowth of nerve elements. Twenty days after the operation, staining the chamber with methylene blue revealed that axis-cyl-

inders were growing out from the proximal stump and proceeding toward the peripheral stump of the nerve. On the proximal stump a neuroma had developed and gave the appearance of a whitish bulb from which issued a large number of blue staining bands, suggesting the appearance of a small onion with its attached roots (fig. 4a and b).

About three months after the operation the distal stump after staining showed a rich supply of axis-cylinders in every way like those seen in the stained normal nerve. Undoubtedly they would have been seen much earlier had the ear been stained. At this time there was apparently complete return of sensory function which probably would have been found present much earlier had tests been made.

Series 5. Grafting of nerves. In order to observe the effect of the insertion of various kinds of grafts into defects made in the auricular nerve many experiments were necessary, some of which will next be considered.

a. *Autografts* (1 expt.). In this experiment a piece of the posterior auricular nerve was resected, turned end for end and placed in the defect created by its removal. As in all the other experiments, the graft was soon invaded by blood vessels and after fifteen days the graft could not easily be distinguished from the rest of the nerve, so effectively had the repair of vascular damage been made. Little change of the vascular supply of the graft occurred during the two weeks that followed. Further observation was prevented by the death of the animal.

b. *Homografts, fresh* (6 expts.). In these experiments the nerves of the right or left ear of two rabbits were exposed and a section (about 5 mm.) of the auricular nerve of one was removed and placed in the defect made in the auricular nerve of the other and vice versa. The complete vascularization of the homografts proceeded as described for the autograft. There did not appear to be an untoward reaction of the tissues of the ear to these grafts. The newly developed blood vessels appeared to have reached their definitive form in about fourteen days as in the autografts. The desirability of perfect alinement of the graft with the proximal and peripheral stumps of the nerve was demonstrated in this series of experiments. In two instances the alinement was poor owing to displacement of the graft when the upper disk of the chamber was applied. In these cases axis-cylinders were not found passing through the body of the graft but were observed outside it. In contrast, in the experiments in which there was more perfect alinement, the axis-cylinders appeared to pass through the body of the graft. Proper alinement of the graft is facilitated by the use of a reading glass and the application of 20 per cent solution of acacia glue—which assists in holding the graft in place. In one animal axis-cylinders had grown through the graft in about seventy days. Vigorous response followed stimulation of the anterior lateral margin of the ear thirty days later.

c. *Homografts, preserved* (8 expts.). Auricular and peroneal nerves were fixed and preserved in 10 per cent solution of formalin for indefinite periods. When desired for use they were soaked for twenty-four hours in several changes of water. They were then placed in 95 per cent alcohol for twelve hours, after which they were left in physiologic saline solution for a few hours before being used. The grafts were 5 to 10 mm. long. An attempt was made to cut the graft long

enough to abut at each end against the cut ends of the auricular nerve. Care was also taken to place the graft in as nearly perfect alinement as might be. This part of the operation was done under a large reading glass.

It required from seven to fourteen days longer for the preserved grafts than for the fresh grafts to become completely vascularized. Ultimately, they were incorporated in the auricular nerve in the same manner as the fresh homografts and the axis-cylinders traversed them successfully, but the time necessary for their growth appeared to be greater since it required as much as five months in one experiment for complete repair and restoration of function.

d. *Grafts protected with cellophane.* In two experiments cellophane was placed under the proximal and distal ends of the nerve and under the graft. It was thought that invasion of connective tissue might be controlled or at least reduced by a cellophane barrier. Whether this was the case was not settled. However it was found that blood vessels grew into the graft from the proximal end in abundance. The number of experiments was insufficient to justify any further statement at present.

Series 6 (4 expts.). In the experiments now to be described the transparent chambers were applied as described in series 2. After complete recovery from the operation the tissues of the chamber were stained and the condition of the nerve was determined. If the preparation was in every way satisfactory, the next step was to section the auricular nerve near the base of the ear. This was done quickly and easily while the area was under infiltration anesthesia with procaine. Subsequently the tissues of the chamber were stained at intervals of seven to fourteen days to determine the progress of regeneration, if any. Under the conditions of these experiments the axis-cylinders after degeneration must make connection with the peripheral stump and traverse a distance of 5 to 8 cm. before they can be seen in the transparent chamber. Eight experiments of this nature are now in progress. All that can be said at present is that the nerves fail to stain with methylene blue about a week after the nerve is sectioned.

We propose to place grafts in defects made in the posterior auricular nerves near the base of the ear and compare the rate of regeneration and repair with results obtained in experiments in which the nerve was crushed or sectioned.

COMMENT. In all of the experiments done thus far we have observed the formation of only one neuroma and that occurred on the proximal stump of a nerve, a piece of which had been resected. Apparently the presence of a graft in some way prevented the formation of neuromata. It will be interesting to observe the results of future experiments in this respect.

Perhaps the most significant observation in this study was that concerned with the development of the circulation in connection with the repair of the injured nerve. It is reasonable to expect the development of the circulation to precede the repair of the nerves and it is important to demonstrate that such is actually the case. The embryologic development of the peripheral circulation precedes that of the peripheral nervous system and regeneration of the peripheral nerves of the adult animal apparently passes through the stages which the nerve passed through during embryonic life. It may be assumed from what has been said of

the importance of the circulation in the regeneration of the nerves that every effort should be made to provide an adequate blood supply for injured nerves in order that their repair may be facilitated. According to a recent indirect report of the work of certain Russian physiologists, they have made observations similar to those we have just described: "The great rôle of blood vessels was ascertained, which were shown to change and develop within the damaged area of the nerve stem" (5). The report also stated that a method was devised for observing the regeneration of nerves in a live animal by means of vertical microscopic illumination. Evidently the Russian workers have also observed what we have seen with respect to the ingrowth of the blood vessels into injured nerves.

SUMMARY AND CONCLUSIONS

Observations have been made in vivo of the repair of the posterior auricular nerve of the rabbit after injury had been done to the nerve in a variety of ways. By the use of transparent chambers and intravital staining, injury and repair of nerves could be observed. The transparent chamber and its application to the ear are described.

Nerves were observed subsequent to crushing, sectioning and grafting with fresh autografts as well as with fresh and preserved homografts.

Regardless of the nature of the injury to which the nerves were subjected, the first occurrence thereafter was the development of a complex network of blood vessels which formed in the crushed region, or which spanned the gap between the proximal and distal stumps when the nerve was sectioned or when a part of the nerve was resected. Similarly when a graft was placed in the course of the nerve, it was first completely vascularized whether it was an autograft or a fresh or preserved homograft. Repair of the nerves occurred more rapidly when fresh than when preserved grafts were used.

The results of the work presented strongly emphasize the importance of the circulation in the regeneration of nerves and suggest that after injury to nervous tissue every effort should be made to provide an adequate blood supply for the injured nerves in order that their repair may be facilitated.

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A COMPARATIVE STUDY OF MUSCLE ATROPHIES CAUSED BY DENERVATION AND ACUTE INANITION¹

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A number of conditions such as denervation, acute inanition, immobilization and tenotomy lead to extensive and rapid atrophy of skeletal muscle. The basic causes for the numerous changes which take place in muscles undergoing atrophy in the above conditions are not well understood. The atrophies due to denervation, immobilization and tenotomy have at least one factor in common, namely, that of a failure of the muscle fiber to develop tension. Scant consideration has been given to the possibility that lack of tension development may also play an important rôle in the muscle changes accompanying acute inanition. Carey (1) has concluded chiefly from morphologic studies on the intercostal muscles of rats that acute inanition is accompanied by a progressive denervation or uprooting of motor nerves which results in a structural detachment of a portion of the motor fibers. This report is concerned with a comparative study of the functional characteristics of muscles undergoing atrophy from acute inanition and denervation and also includes observations on the state of neuromuscular transmission in intact and regenerating nerves of fasting animals.

EXPERIMENTAL METHODS. The studies were carried out on the gastrocnemius muscles and tibial nerves of adult albino rats. The animals were placed in individual cages and deprived of all food except water. The duration of the longer fast periods averaged 11 days during which time the animals lost about one-third of their body weight. The controls consisted of animals from the same stock and of the same age group and had access at all times to their usual diet. In a number of animals complete denervation of one gastrocnemius muscle was accomplished by crushing the tibial nerve with a heavy ligature. The nerve and muscle of the contralateral unoperated limb were used as controls. A period of 21 days was allowed for the partial regeneration of the nerve. Some of these operated animals were fasted for the last 11 days of this period. Other operated animals were non-fasted and served as controls for this group.

The strength of the muscles was determined by measuring the maximal isometric tension which developed in response to volleys of slightly supermaximal stimuli applied to the tibial nerve and directly to the muscle. The techniques employed for stimulation and strength measurements have been described in detail elsewhere (2). Following the strength measurements the muscles were dissected, weighed and analyzed for creatine.

A search was made for the presence of fibrillary activity in the muscles of fasting animals by amplification of the action potentials led from needle electrodes buried in the intact muscle and by visual observation of the exposed

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muscle. In order to check on the adequacy of the electrodes and amplification system the tibial nerve of one limb was sectioned 3 to 4 days before the termination of the fast. This made it possible simultaneously to compare the action potentials in the contralateral muscles of an animal, one muscle subjected to fasting, its contralateral member to combined denervation and fasting.

Attempts were made to ascertain whether the muscles from animals subjected to prolonged fasting exhibited an increased sensitivity to acetyl choline comparable to that displayed by denervated muscle. The tests of acetyl choline sensitivity were made upon rats which had been fasted for a period of 11 days. In order to provide a control for the effectiveness of the injection techniques, the tibial nerve of one limb was sectioned 4 to 5 days before the tests were made. The gastrocnemii were exposed and the tendons attached to light isotonic levers which recorded on a kymograph. The animals were kept under light

TABLE 1

A summary of average values and standard errors for muscle creatine and strength elicited by direct and nerve stimulation

DURATION OF FAST	NUMBER OF ANIMALS	PER CENT LOSS OF BODY WEIGHT	CREATINE PER 100 GMS. MUSCLE		$\frac{\text{TENSION (NERVE*)}}{\text{TENSION (MUSCLE*)}} \times 100$	$\frac{\text{GMS. TENSION}}{\text{GMS. BODY WEIGHT}}$
			Fasted	Denervated†		
hours			mgm.	mgm.		
0	76	0			91.8 \pm 1.0	9.70 \pm 0.16
0	6	0	450	460	92.0 \pm 1.7	9.40 \pm 0.42
72	6	10.8	461		91.5 \pm 2.5	10.00 \pm 0.42
144	6	19.2	470	437	95.3 \pm 2.9	9.05 \pm 0.54
222	6	28.8	473	414	93.9 \pm 4.7	8.63 \pm 0.96
240	22	31.0			92.4 \pm 1.8	8.27 \pm 0.49

* Stimulation.

† At degrees of atrophy comparable to that of the weight loss in muscles of fasting rats.

anesthesia and given 0.1 mgm. atropine sulfate and 0.1 mgm. eserine sulfate a few minutes before the intravenous administration of 1 mgm. acetyl choline.

RESULTS. Under the conditions of our experiments the ratio of the tension developed by the gastrocnemius muscles of normal control rats in response to nerve stimulation to that elicited by direct muscle stimulation was quite constant (table 1). This ratio was not altered even in the terminal state of a prolonged fast. This finding indicates that prolonged acute inanition did not lessen the capacity of the motor nerve to activate its muscle and offers evidence against any functional denervation of muscle during prolonged acute inanition. Table 1 summarizes the average values for the strength of the gastrocnemius, when measured by the tension which developed in response to nerve stimulation and expressed as total grams of tension per gram of body weight. It is to be noted that even in the terminal stages of a fast, motor nerve stimulation elicited in the gastrocnemius muscle a tension response per unit of body weight which was only slightly less than the values observed in the muscles of well fed animals. The loss in strength in the gastrocnemius muscle during acute inanition was

found to parallel closely the loss in total body weight. The loss in contractile strength per unit of muscle cell phase was found to be much greater in denervated muscle than in fasting muscle undergoing comparable amounts of weight loss (2). Muscles from fasting animals exhibited an increased creatine concentration, while muscles previously subjected to denervation contained a lower concentration of creatine.

The data (table 2) on the tissues from animals subjected to the denervation operation indicate that the regenerating nerve fibers of fasting animals were able to make functional contacts with muscle units quite as effectively as those of well nourished animals. The ratio of the isometric tension developed by muscles at 21 days after denervation to that developed by their unoperated contralateral controls was precisely the same in fasting as in well nourished animals. This was true for both the response to stimuli applied directly to the muscle and to indirect activation through the regenerating nerve. Likewise the degree of atrophy at this time as determined by the relative weights of muscles

TABLE 2

Summary of average values and standard errors for control and fasted animals

Tests were made at 21 days after tibial nerve crush. The fasted animals were without food for the last 11 days.

CONDITION	NUMBER OF ANIMALS	AGE	PER CENT LOSS OF BODY WEIGHT	WEIGHT OF DENERVATED MUSCLE*	RELATIVE STRENGTH OF DENERVATED MUSCLE* WHEN ACTIVATED THROUGH	
					Nerve	Muscle
Control.....	17	107		64.4 \pm 1.5	31.9 \pm 2.5	50.9 \pm 2.3
Fasted.....	11	119	32.6 \pm 3.3	65.1 \pm 2.4	32.8 \pm 3.2	48.9 \pm 2.8

* Expressed as per cent of that found in non-denervated contralateral control.

from operated and non-operated limbs was essentially the same in fasting and non-fasting animals.

Many attempts were made to ascertain the presence or absence of fibrillary activity in muscles of animals subjected to acute inanition. The techniques employed were such as consistently to reveal their presence in the denervated muscles of control and fasting animals. However, in no instance was evidence found for the presence of fibrillary contractions in the gastrocnemius muscles of fasting animals. This was true even in the terminal states of acute inanition.

Under the conditions of our experiments denervated muscle consistently develops a contracture response to less than 1 mgm. of intravenously injected acetyl choline (3). However, 1 mgm. of acetyl choline consistently failed to evoke any contracture response in non-denervated muscles of fasting animals under conditions which permitted a typical response in the muscles of the contralateral denervated limbs. Our findings do not establish the fact that muscles from fasting and non-fasting animals exhibit the same degree of sensitivity to acetyl choline injections but rather that fasting muscles do not display an increased sensitivity comparable to that of denervated tissue.

DISCUSSION. These investigations point to a normal state of neuromuscular relationships during acute inanition and are opposed to the concept that a "fasting denervation" exists in such a condition. If any appreciable decrease occurs in the tension developed by skeletal muscle during acute inanition, it must be ascribed to factors other than the capacity of motor nerve to activate its muscle. Although for technical convenience our studies were made upon the gastrocnemius muscles while those of Carey (1) were made upon the intercostal muscles, it is not apparent that any fundamental differences should exist between these two muscle groups in their responses to acute inanition. We were unable to find that muscles from fasting animals exhibit any of the typical characteristics of denervation atrophy. The increased sensitivity to acetylcholine injections and the constant presence of fibrillary contractions are highly specific and sensitive criteria for the state of motor denervation. These were consistently absent from the gastrocnemius muscles of fasting rats. Moreover important differences were noted between denervated and fasting muscles with respect to creatine concentration and strength. These findings together with the observation that nerve stimulation elicits the same amount of tension in muscle relative to that from direct activation in both fasted and well nourished animals argue against any significant denervation or trophic estrangement of muscle and nerve in acute inanition. Whatever the functional significance of the morphologic changes in neuromuscular terminations described by Carey (1) may be, it is apparent that the changes taking place in muscle during acute inanition cannot be attributed to denervation.

SUMMARY

A comparative study was made of atrophies due to acute inanition and denervation in the tibial nerves and gastrocnemii of albino rats. No evidence was found that prolonged acute inanition resulted in "functional denervation" of skeletal muscle. The muscles of fasting animals did not exhibit characteristics typical of denervation atrophy, such as the presence of fibrillary activity and the contracture response to acetylcholine injections. Nerve stimulation elicited the same amount of tension relative to that from direct activation in both fasted and well nourished animals. The regenerating nerve fibers of fasted animals were able to make functional contacts with muscle units quite as effectively as those of well nourished animals.

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THE EFFECT OF ANOXIA ON PERISTALSIS OF THE SMALL AND LARGE INTESTINE¹

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In 1934 Schnohr (1) reported studies on the effect of anoxia on intestinal peristalsis in rabbits. He inserted an oval cellophane window in the abdominal wall and observed that anoxia or an increase in CO₂ concentration in the blood caused an immediate cessation of all intestinal movement.

Peterson, Smith and Hale (2) in 1938 using a modification of Macht's technique studied the effect of CO on gastrointestinal peristalsis in rats. They reported that when the blood was saturated from 70-80 per cent with CO, gastrointestinal peristalsis was inhibited 33 per cent. "The egestion time" by which they meant the time required for the first appearance of fecal pellets following the test meal was prolonged 22 per cent. Emerson (3) in 1937 using Macht's technique and also working with rats reported that surgical anesthesia with ether inhibits the motility of the small intestine for the period of its duration. By the second hour after anesthesia was terminated normal activity of the intestine was regained.

No quantitative data have been published of the effect of anoxic anoxia on the peristalsis of the small intestine. So far as the authors are aware, moreover, no studies ever have been reported to show what effect, if any, anoxia has on the movements of the colon. It was deemed that such studies were timely.

METHODS. *The small intestine.* The animals used in this study were dogs and mice. Essentially, Macht's technique was employed in these investigations.

Dogs. Matched pairs of dogs were used, one animal to serve as control, the other as the experimental animal. They had had no food for 24 hours previous to the experiment. About 40 cc. of a mixture of 10 per cent charcoal suspension in 10 per cent gum acacia in water was given by stomach tube. Eight minutes following intubation the experimental animal was placed in a low pressure chamber and subjected to oxygen want. The accumulation of CO₂ in the chamber was prevented by providing for adequate ventilation. The control animal was placed in the immediate vicinity of the chamber. Both animals were subjected to the same temperature and all factors were controlled as carefully as it was possible to do.

At the end of 30 minutes the animals were allowed to breathe a fatal concentration of ether, the small intestines were removed, slit open and the distance which the charcoal mixture had traversed the intestine was measured with a meter stick.

¹ Aided by a grant of the Ella Sachs Plotz Foundation.

The following partial pressures of oxygen were used: 80, 63, 53 and 43 mm. Hg corresponding to atmospheric pressures of 379, 294, 246 and 206 mm. Hg and to approximate altitudes of 18,000, 24,000, 28,000 and 32,000 feet respectively.

Mice. Macht's technique was also applied to young adult mice. Groups of 5 mice were rapidly given intragastric injections of 1 cc. of 10 per cent charcoal suspension in 10 per cent acacia and (10 min. later) were placed in the low pressure chamber. An equal number of controls were used. The mice were removed from the chamber at the end of thirty minutes, that is, 40 minutes after intubation and killed by a blow on the head. The control mice too were sacrificed in the same way at the end of 40 minutes. The small intestines were removed and the distance the charcoal mixture had traversed was measured. Three partial pressures of oxygen were used: 94, 80 and 47 mm. Hg corresponding to atmospheric pressures of 446, 379 and 225 mm. Hg and to approximate altitudes of 14,000, 18,000 and 30,000 feet respectively.

TABLE 1
The effect of anoxia on peristalsis of the small intestine in mice

pO ₂	ALTITUDE	CONTROL			ANOXIA			"p"
		Number of animals	Length of gut	Cm. of gut traversed at end of 40 min.	Number of animals	Length of gut	Cm. of gut traversed at end of 40 min.	
mm. Hg	feet							
94	14,000	20	47.1	42.6	20	46.6	39.4	>0.1
80	18,000	25	44.3	39.7	25	44.4	30.5	<0.001
48	30,000	25	43.4	39.1	20	44.7	24.9	<0.001

* According to Fisher.

The colon. Only dogs were used when the colon was studied. They were given 300 mgm. of sodium barbital intravenously. The colon was exposed and tracings were taken of the movements of the longitudinal muscles and also of the circular muscles. They were not, however, taken simultaneously. The movements of the longitudinal muscles were taken by means of an enterograph described by Lawson (4). The movements of the circular muscles were recorded by simply inserting into the colon a small balloon, which was attached to a Marey tambour. The balloon was distended to a pressure equivalent to about 8-10 cm. of water. Anoxia was produced by diluting oxygen with nitrogen to the desired percentage, and administered by an appropriate apparatus described elsewhere (5).

RESULTS. *The small intestine.* The results are shown in tables 1 and 2. The data indicate that intestinal activity in mice is not materially affected at a partial pressure of oxygen of 94 mm. of Hg, but if lower partial pressures are used, there is a statistically significant decrease in intestinal movements.

In expressing the results obtained in mice the actual distance the charcoal traversed was used, because the average gut lengths in control and experimental

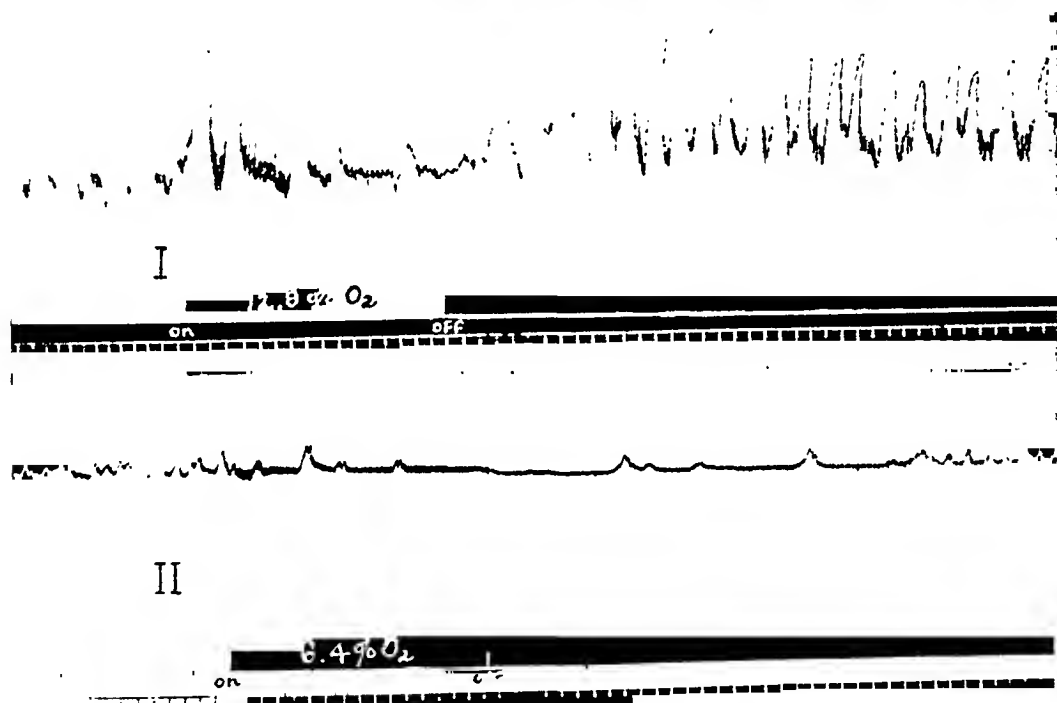
animals were substantially the same with but little individual variation. In the dogs although an earnest attempt was made to select matched pairs there was considerable individual variation in the gut lengths and it was, therefore, deemed best to use percentage figures.

Anoxia apparently did not affect the intestinal activity of dogs. Even when extreme grades of anoxia were employed (partial pressure of oxygen of 43 mm.

TABLE 2

The effect of anoxia on peristalsis of the small intestine of the dog

pO ₂ (mm. Hg)	159	80	63	53	43
Altitude (feet)	0	18,000	24,000	28,000	32,000
Number of animals	37	3	4	10	22
Mean distance traversed (per cent)	65	62	77	71	54
Standard deviation	22.5	11.5	10.1	25.3	17.2
Range	20-94	50-73	63-85	24-95	28-91



Figs. I and II

Hg) intestinal activity in the anoxic animals did not differ significantly from the controls.

Figure I shows the effect of anoxia on the contraction of the longitudinal muscles of the colon. It will be noted that 12.8 per cent of oxygen practically abolished colonic contractions. There was practically no change in tone. Figure II which illustrates the contractions of the circular muscles of the colon shows that anoxia also causes a diminution of the contractions of these muscles.

The threshold for both longitudinal and circular muscles lies from about

14-12 per cent oxygen, which corresponds approximately to partial pressures of 110 and 94 mm. Hg and to simulated altitudes of 10,000 and 14,000 feet respectively.

DISCUSSION. It has been shown previously that an effective degree of anoxia is capable of decreasing hunger contractions (6) and digestive peristalsis (7). It has been shown also that anoxia may delay gastric emptying in both man (8) and dog (9). It is believed that the small intestine has the same innervation as the stomach. In view of this it would be expected that the movements of the small intestine would likewise be inhibited by anoxia.

The data which we present are obviously not in entire accord with the results reported by Schnohr (1) who found that anoxia produced an immediate cessation of intestinal movements. Our observations on dogs indicate that anoxia had no effect on intestinal peristalsis, and mice even when subjected to severe grades of anoxia showed only a partial inhibition of intestinal peristalsis. Schnohr used rabbits, but there is no reason to believe that these animals would react differently to anoxia than other mammals.

It is of interest to comment on the intestinal peristalsis seen in the guinea pig under certain conditions. If this animal be struck a blow on the head and if its abdomen be opened at once, the intestines often will show a vigorous peristalsis. This stimulation presumably is brought about by the fulminating anoxia, which in this instance is both of the anoxic and anemic type. It may be that under these conditions the two types of anoxia are synergistic to each other. Carbon dioxide, of course, is also present. This pronounced increase of intestinal peristalsis, however, is only transitory in nature and the intestines soon become quiescent. This increase in peristalsis produced by the fulminating anoxia, however, is quite a different phenomenon than that brought about by prolonged anoxia of much less severe grade.

There is considerable evidence that anoxia stimulates the sympathetic nervous system, and causes liberation of epinephrine from the adrenal glands. Since the inhibitory fibers to the small intestine are carried by the sympathetic division of the autonomic nervous system it would be expected that anoxia would inhibit the small intestine in the dog as it does the stomach. We can offer no explanation why the small intestine of the dog did not respond to anoxia.

In order to ascertain whether the method we used to determine intestinal motility in dogs was satisfactory, a number of animals were intubated and given the charcoal mixture previously described and ephedrine (25 mgm./kgm.) was given subcutaneously 3 minutes later. This preparation produced a statistically significant decrease in the propulsive type of peristalsis of the small intestine. It is of interest to point out that ephedrine produces a marked delay in gastric emptying (10) and thus has an effect similar to anoxia.

In another series of animals prostigmine (0.5 mgm./kgm.) was administered also subcutaneously following intubation. These animals showed a statistically significant increase in intestinal peristalsis. Due to the results obtained by the use of ephedrine and prostigmine it was concluded that our method of determining intestinal motility in the dog was a workable one.

The accompanying graphs clearly show that anoxia produces a diminution in the height of contractions of both the longitudinal and circular muscles of the colon. In most instances following the period of anoxia a supernormal phase intervened; this, however, was not always manifested. Anoxia generally caused a loss of tone, although occasionally no change in tone was noticeable. An occasional animal was quite resistant to anoxia, but in the average dog the threshold value was found to be about from 14 to 12 per cent oxygen which corresponds to a simulated altitude of 10,000 to 14,000 feet respectively.

It often has been stated that the colon does not have as rich an arterial blood supply as does the upper part of the gastrointestinal tract. If this is true, it might be expected that the colon would be more resistant to anoxia than would the stomach or small intestine. In most animals, however, colonic movements were quite sensitive to oxygen want. In no case, moreover, did anoxia actually stimulate contractions of the colon or cause defecation to take place. It is of interest to mention that although defecation did not take place while the animals were under barbital anesthesia unanesthetized dogs often defecate when they are subjected to severe grades of anoxia. It is possible that the anesthetic agent depressed the defecation reflex.

SUMMARY

The effect of anoxia was studied on the activity of the small intestine in normal mice and dogs. Essentially Macht's technique was employed. It was found that partial pressures of oxygen of more than 94 mm. Hg had no effect on the intestinal motility of mice; lower partial pressures caused a statistically significant decrease in intestinal motility. The motility of the small intestine of dogs was unaffected by anoxia within the ranges used (partial pressures of oxygen from 80-43 mm. Hg).

The effect of anoxia was also studied on the motility of the colon of barbitalized dogs. Anoxia produced a diminution in the height of the contractions of both the circular and longitudinal muscles. In the average dog, activity was first decreased at partial pressures of oxygen from 110-94 mm. Hg corresponding to approximate altitudes of 10,000 to 14,000 feet.

We wish to express our best thanks to Dr. Hampden Lawson for the use of his enterograph.

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THE EFFECT OF SEVERE HEMORRHAGE, SEASONAL TEMPERATURE AND DIURNAL VARIATION ON BLOOD LACTATE IN THE DOG^{1, 2}

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The blood lactate response in the dog following an acute, severe hemorrhage exhibits a quick rise, followed by a slow decline, and reaches by the end of the first twenty-four hour period after the hemorrhage a level at, or below, the pre-hemorrhage value. This work was first established by Riegel (1927).

Yamada (1937) subjected mature, healthy rabbits to carefully controlled temperature for varying periods of time. When the temperature was 40°C. or 50°C., he noted a considerable increase in blood lactate. Although the animals remained in the high temperatures from thirty minutes to one hour, it required some three to six hours for the blood lactate to return to the resting level. Truka-Tuzson (1940) made ten to twenty determinations of blood lactate per month on different persons throughout a nine-month period from September through June. His lactate values as expressed by the monthly mean varied directly with the monthly mean temperature. Dill *et al.* (1940) found an increase in concentration of blood lactate of approximately 50 per cent when determined in Benoit, Mississippi, as compared to the values found in the same human subjects in Boston, Massachusetts.

An attempt has been made to ascertain if there is a diurnal variation in the blood lactate in the normal, resting dog. In addition, a study has been made of the variation from dog to dog and of the possibility of using some central figure as a norm in referring to the expected blood lactate concentration. In table 1 are given some representative values of blood lactate taken from the literature and other sources. It is interesting to note the extreme variation in values, not only from author to author but also from the low to the high values given by any particular author. The percentage variation, in all but one case, is at least 100 per cent.

¹ This work submitted as partial fulfilment of the requirements for the degree of Doctor of Philosophy in Physiology at the University of Kansas.

² A preliminary report of part of this work was presented before the American Physiological Society in 1941; This Journal 133: P466, 1941.

Such variations are not the only ones which ought to be considered when evaluating the data dealing with blood lactate in relation to physiological and experimental manipulations. The literature contains little reference to any extended investigation of the blood lactate concentration with respect to the variation which might be expected in an unanesthetized, resting subject from time to time, and the regularity of such variation, *i.e.*, diurnal variation.

The present investigation attempts to answer the following questions: (1) Is there a secondary, delayed hyperlactacidemia following severe, acute hemorrhage? (2) What time relationships are exhibited by the blood lactate response? (3) Is the blood lactate of healthy dogs affected by seasonal changes in tem-

TABLE 1
Typical normal blood lactate values from various authors

AUTHOR	NUMBER OF SAMPLES	VALUES		
		Low	High	Average
Man				
		<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>
Clausen (1922).....	3	21.8	32.1	28.2
Barr, Himwich and Green (1923).....	6	14.0	25.2	19.2
Long (1924).....		10.0	20.0	
Schultze (1926).....		9.0	13.0	11.0
Dog				
Gaglio (1896).....		17.0	157.0	
Collazo and Lewicki (1925).....	3	19.6	134.0	58.5
Houget (1933).....	13	10.0	69.0	32.0
Binet and Klukowski (1933).....	66			28.0
Edwards, Brouha and Johnson (1938).....		5.4	14.6	
Ivy and Crandall (Personal communication)....	135	6.0	25.0	12.9
Swan (Present study).....	234	2.0	13.3	10.8

perature? (4) Is there a diurnal variation in the blood lactate in resting, healthy dogs?

To determine the effect of severe hemorrhage on the blood lactate three male and three female dogs were kept in individual cages throughout the experiment. Feeding and watering were done at scheduled times. All samples of blood were withdrawn at the same hour on each sampling day. Complete resting conditions were maintained for each animal. Two additional animals—one male and one female—were used as controls.

To establish basal blood levels on the six experimental animals, samples were taken between 7 p.m. and 9 p.m. on at least two successive days before the hemorrhage. These samples were 6 cc. amounts.

Hemorrhage was achieved by cardiac puncture while the dog was held by an assistant. From 25 to 32 per cent of the total calculated blood volume was

removed in each case. No samples were taken after the hemorrhage until at the expiration of the first twenty-four hours for five days. Thereafter, samples were taken every forty-eight hours through the ninth day.

In work on the effect of seasonal temperatures on blood lactate, two dogs—a female, no. 9, weighing 10.2 kgm., and a male, no. 10, weighing 24 kgm.—were confined in a small, unheated but fully enclosed house constructed of light wooden sheeting. This structure offered complete shelter from wind, rain and snow but afforded little protection to temperature changes. The inside temperature followed within 1° to 3°C . the outdoor temperature.

The thermometer used was the Bristol Recording Thermometer.³ Continuous temperatures throughout the twenty-four hour period were thus available. This instrument provided readings which served as the semi-official temperatures for the community.

Uniform feeding and watering procedures were used for the dogs. The food was a balanced, commercial product which was given to them once each day at 5 p.m. The dogs' conditions and weights improved during the experiment, indicating a healthy state at all times.

Samples were taken from the peripheral leg veins, all four legs being used in rotation. All samples were taken at 11 a.m. every day of the week except Sundays and holidays. Approximately 12 cc. of blood were removed each time a sample was obtained. That such a blood loss was in no way injurious was indicated by the improvement in each dog's condition and vigor.

To insure a state of complete rest in the experimental animal, both dogs were leashed for at least forty-five minutes previous to the time of blood removal. No form of activity was permitted during this rest period.

The experiment was started early in the fall when the temperature daily reached a peak almost as high as during the hottest part of the summer. Samples were taken every other day from each dog for a period of six months. During this period, temperatures approximating those of hottest summer as well as those of coldest winter were registered.

To determine the effect of diurnal variation on blood lactate, dogs were selected from the general dog pen and allowed to rest for forty-five minutes before the first sample was drawn. Food was withheld from the animal during the twenty-four hour period of sampling. Every precaution was taken to insure conditions of complete rest throughout the entire test period.

Males and females were used on alternate test periods. Insofar as possible, a different dog was used for each test period. However, it was found necessary to use one particular dog—a male—for three test periods.

This series of experiments was continued for a twelve-month period; hence ten different dogs were used. Each test period included twenty-four samples, distributed an hour apart throughout the twenty-four hour period. Each twenty-four hour run was made once a month on approximately corresponding days.

METHOD. Samples of venous blood were withdrawn from peripheral veins into glass syringes containing sufficient dry sodium oxalate to prevent coagula-

³ Courtesy of the Kansas Public Service Company, Lawrence, Kansas.

tion. Exactly 5 cc. were measured by an Ostwald pipette and delivered into diluting flasks. To this measured sample of blood were added 8 volumes of N/12 sulfuric acid and 1 volume of 10 per cent sodium tungstate. Glycolysis was avoided by completing the blood withdrawal and precipitation of proteins within five minutes.

The blood was filtered through paper and the carbohydrates removed by the method of Van Slyke (1917). Then after centrifuging, the tubes containing the filtrates were stoppered and stored in the refrigerator. Within a week the lactate content was determined by the method of Friedemann, Cotonio and Shaffer as described by Peters and Van Slyke (1932).

All samples except those obtained in the work on the effect of diurnal variation were run in duplicate, the results agreeing within 2 mgm. per cent in practically

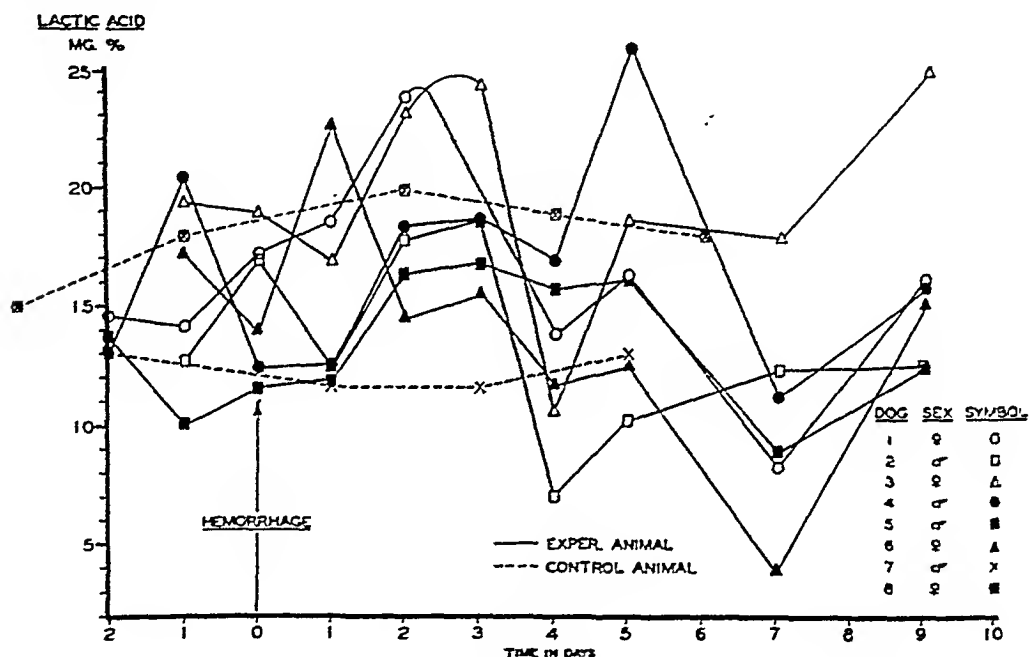


Fig. 1. Effect of severe hemorrhage on blood lactate in dogs

all cases. All blood filtrates were analyzed within a week after removal from the dog and, in the interim, were kept in stoppered tubes in the refrigerator.

RESULTS. *The effect of severe hemorrhage on blood lactate.* In figure 1 a complete record of the data is presented. Examination of the blood lactate values obtained from each dog before hemorrhage indicated that, even in the normal, resting subject, there was considerable variation. This variation was somewhat random in nature. Dogs 1, 2 and 5 showed higher lactate values in the second sample than in the first. On the other hand, dogs 3, 4 and 6 showed a decrease when their second sample was compared to their first.

Forty-eight hours after the hemorrhage, decided increases in blood lactate were found in all dogs except no. 6, a female, whose blood lactate had, by this time, decreased to the basal level. Seventy-two hour samples gave values slightly higher than those of the second day in every case except dog 1, whose sample was lost in the titration.

The blood lactate values obtained on the fourth day were generally lower than any previous results, either normal or after hemorrhage. On the fifth day, values generally higher than on the fourth were found and, in one case, that of dog 4, the highest values for that animal and for the series were obtained.

From this point, samples were drawn every other day. Those obtained seven days after the hemorrhage gave values which, in most cases, were below those previous to the hemorrhage. In all but dogs 2 and 3 the values were the lowest of the whole series. On the ninth day the lactate concentrations had again risen to about the pre-hemorrhage level, although in one dog, no. 3, the concentration was quite high.

While there is considerable variation, yet, in general, it can be noted that the variation before hemorrhage is at random. Twenty-four hours after hemorrhage the lactate levels are, with two exceptions, at the resting level for any given dog.

In five of the six dogs, the lactate concentration was sharply increased at the end of forty-eight hours. Approximately the same high level was present at the end of seventy-two hours. Then, without exception, lower values were found at the end of ninety-six hours. Some of the ninety-six hour values were lower than the pre-hemorrhage levels.

From the ninety-sixth hour to the end of the experiment—where the majority of the levels approached the pre-hemorrhage resting values—the lactate variations occurred in unison. Some variations were of greater magnitude than others, however. Throughout the duration of the recovery period there were periodic fluctuations of considerable extent.

In some way, then, the random behavior of the blood lactate level had been modified by the hemorrhage so that it displayed a periodic nature. It is obviously not totally a response to a deficient oxygen supply to the tissues, even though this may cause a hyperlactacidemia as has been shown by Araki (1891) and many others since. It is difficult to see how the tissues could be adequately supplied with oxygen at the end of twenty-four hours and be inadequately supplied in the forty-eighth hour, although the animal was in a state of rest during both periods.

Doubtless, a hyperlactacidemia during or immediately following hemorrhage, such as Riegel (1927) described, is best accounted for on a basis of oxygen deficiency. Gesell *et al.* (1930) have shown in anesthetized, operated animals that such a hyperlactacidemia is found following a reduction in oxygen content in the respired air. Similarly after hemorrhage, Gesell obtained high blood lactate values. These experiments were of short duration and on animals under conditions not normal. Hence, it may be questionable to attempt a direct transposition of results.

Particularly is this true in view of the experiments of Cook and Hurst (1933). These investigators, using healthy human subjects, found that the lactate content of the blood during rest was the same as during a period of light to moderate exercise. Even when their subjects walked at a rate of three miles per hour no increase in blood lactate could be found in venous blood immediately draining the active muscle groups.

Jervell (1928) in studying the blood lactate concentration in the blood of anemic patients was unable to demonstrate any consistent relationship between hemoglobin percentage or red cell counts and lactic acid. Jervell concluded that deficient oxygenation was seldom, if ever, serious enough in anemic patients to prevent the combustion of the amount of lactic acid normally produced.

Bock, Dill and Edwards (1932) failed to find any significant change in blood lactate of normal men following reduction of inspired oxygen to nine per cent, ingestion of sodium bicarbonate or ammonium chloride or taking moderate exercise. These investigators demonstrated a change in pH and alkaline reserve in some of the above procedures, but they could demonstrate no consistent relationship between hydrogen ion concentration and lactate changes in the blood of their subjects. Summing up their results, in the light of work done by other investigators, these authors remark:

Of the lactic acid present in the blood of a resting subject a small portion may come from muscle activity, the rest presumably from the activity of the central nervous system, various glandular activities, etc. It seems logical to suppose that the ability to reconvert lactic acid to its precursor may vary greatly at the seat of its formation, just as the rate of utilization of oxygen may vary from organ to organ in the body. The problem remains for study along lines differing from those suggested in the past.

It is our opinion, too, that the level of blood lactic acid in the resting subject must be accounted for in a way which differs from any suggested previously. Therefore, we venture such a suggestion.

A possible explanation of the blood lactate response is afforded by linking the lactate level of the subject, whether in ordinary rest or rest following severe hemorrhage, with the showers of leucocytes which are liberated into the blood stream. The evidence, at present, is only suggestive since there is little in the literature concerning extended observations of the leucocyte count as related to other physiological variables. The observations which have been reported, although fragmentary, indicate that there may be some relation between the presence of leucocytes and lactate concentration in the blood of resting subjects.

This is not to be construed as denying the well established fact that blood lactate varies under conditions of physical stress. Our purpose is to call attention to some similar aspects of leucocyte and blood lactate changes.

Drinker, Drinker and Kreutzmann (1918), in one of the few extended experiments on the cellular content of the blood of dogs following large hemorrhage, showed that the discharge of leucocytes into the peripheral blood followed a pattern which exhibits many similarities to the lactate variations in our experiments.

We wish to emphasize that there is no direct relation between our blood lactate and Drinker, Drinker and Kreutzmann's leucocyte values. Such agreement is not to be expected since the two variables were determined on different dogs at widely separated times. The experimental conditions were different, since leucocyte counts were obtained during a study of the effect of repeated hemorrhages and saline infusions under anesthesia, while our lactate values were taken following one severe hemorrhage on normal, resting dogs.

The effect of seasonal temperature on blood lactate. A complete record of the data collected in the experimental period of six months shows considerable variation from one determination to the next, even in the series from the same animal. Even though there is great variation from one sampling day to another, yet this variation is no greater than is the variation observed when several successive samples are taken from a dog during a twenty-four hour period (unpublished work). In addition to the greater variability, dog 9 consistently contained a higher concentration of blood lactate than did dog 10.

There is no relation between the blood lactate changes represented and the day-by-day temperature changes. Neither is there a direct relation between

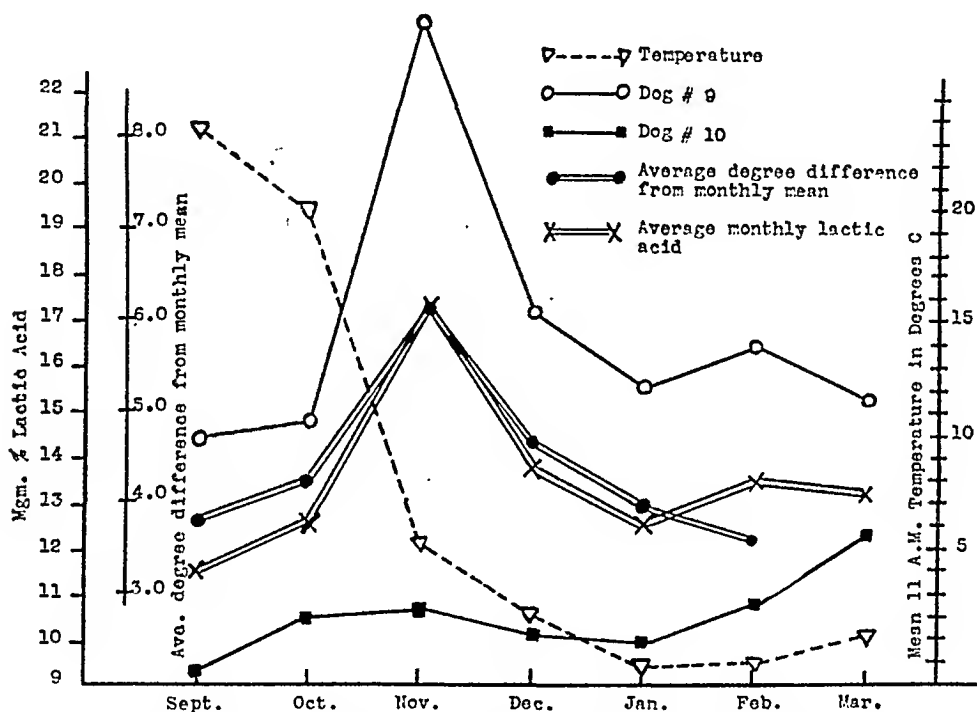


Fig. 2. Average values for blood lactate and temperature. The lactate curve represents monthly mean values. The temperature curve represents the dispersion of the daily 11 a.m. temperature from the monthly mean 11 a.m. temperature.

the average lactate values for a given month and the average 11 a.m. temperature during the month.

However, a general trend in the same direction with respect to both dogs was observed. This similarity indicated that the changes in blood lactate were brought about by the same common agent or agents. But any such blood lactate response may be modified, no doubt, by a multiplicity of factors.

Examination of figure 2 shows a close correlation between the average monthly blood lactate values and a variable which we have chosen to designate the "average degree difference from the monthly mean." This quantity and the statistical mean deviation are identical. It is a measure of the dispersion of the daily 11 a.m. temperature from the mean 11 a.m. temperature for a given month. Surprisingly close agreement is observed between these two variables for the

experimental period. Such behavior is interpreted to mean that results in a blood lactate change but more probably the temperature fluctuates around a central point.

Perhaps, too, a total temperature change may influence the blood lactate, since the February lactate values were higher for both dogs than the mean deviation would seem to warrant. Therefore, it may be that, in addition to the degree of variation as a factor, a large temperature change of a continuous nature was an additional factor. With respect to the March values, it is seen that there is some disagreement here, too. It is felt, however, that too few values are represented upon which to base any definite conclusions.

The effect of diurnal variation on blood lactate. The data indicated that there were two distinct periods of high concentration during a given twenty-four hour test. One high tide occurred about noon and the other about twelve hours later, i.e., near midnight.

The mid-day rise began near 10 a.m., reached a peak at 12 noon, and then subsided to below the mean value for the group by 4 p.m. The mid-night rise was quite pronounced, but it lacked the well sustained character of the mid-day increase. Its peak was a little higher than the mid-day peak, yet it exhibited a somewhat fluctuating nature, since the 12 midnight value is lower than the 11 p.m. or the 1 a.m. values.

One transient rise occurred about 6 p.m. It appeared and disappeared quickly although its magnitude was almost equal to either of the two previously described.

Lactic acid in the blood of the resting organism has been considered the result of acid production by contracting muscles and its subsequent diffusion into the blood stream. Recent experimental work on man failed to account for more than a part of the resting blood lactate as due to such a course. Bock, Dill and Edwards (1932) suggest that resting blood lactic acid "May be a split product of carbohydrate mobilized for the maintenance of the general metabolism of the body." This generalization is so vague that it offers little aid in explaining the facts.

A comparison of the behavior of blood lactate with the behavior of other constituents of the blood under conditions of complete rest reveals the interesting fact that the only constituent in the blood which exhibits diurnal variations of comparable frequency and magnitude is the white blood corpuscle. Further study of the concentration of these structural elements suggests a striking similarity to the concentration of blood lactate.

One careful twenty-four hour study of the diurnal variation of leucocytes was that of Shaw (1927), in which he followed the leucocyte changes in four normal human subjects by performing white cell counts every hour throughout a twenty-four hour period. Sabin *et al.* (1925) studied the normal rhythm of the white cells in resting men covering the daylight hours of 9 a.m. to 4 p.m. only. Tschishikow (1927) studied the so-called digestion leucocytosis in dogs and included a curve showing the rhythm of the leucocytes in resting fasted dogs during the period from 8:20 a.m. to 9:30 p.m. Zirm and Bauermeister (1933) followed

the leucocyte variations on resting hospital patients and obtained results quite similar to those published by Shaw (1927).

These authors obtained evidence of diurnal variations in the leucocyte count. When their values were plotted the resulting curves resemble the curves showing lactic acid changes. In all studies there was a mid-day rise in leucocytes with considerable variation from hour to hour. In fact, Sabin spoke of an "hourly rhythm of leucocytes." In the more extended studies there was also a mid-night swell. The late afternoon rise was present in Shaw's series but came an hour later in Tschishikow's.

The diurnal variations in leucocytes in man along with the blood lactate mean hourly values of our resting dogs are strikingly similar in their time relationships but one must bear in mind that they represent different species.

This marked similarity may be merely an accidental occurrence. Without direct evidence concerning the relationship of the two variables, one would be unjustified in assigning to one the rôle of cause and to the other that of effect, or to both the result of a common cause.

An attractive hypothesis would be to consider the blood lactate in the resting organism, partly at least, the result of the glycolytic activity of the white cells.

It is well known that one of the potentialities of the white cell, particularly the segmented cells, is the production of lactic acid from carbohydrates. Levene and Meyer (1912) first demonstrated leucocytic splitting of dextrose in vitro. Maclean and Weir (1915) demonstrated that both erythrocytes and leucocytes glycolyzed dextrose in vitro but that the activity ratio of leucocytes to erythrocytes, cell for cell, varied roughly from 200:1 up to 1000:1 in favor of the leucocytes. They concluded that, in normal blood, the white cells probably exert greater glycolytic effect than the red cells even though they are present in much smaller numbers. Falcon-Lesses (1927) proved that leucemic blood in vitro displayed greater glycolytic activity than normal blood.

DISCUSSION. *The effect of severe hemorrhage on blood lactate.* The significant point to be observed concerning the relationship of the delayed hyperlactacidemia and leucocyte increase following severe hemorrhage in dogs is that the direction and rate of variation of the two are similar. They simply show that, when an animal is subjected to a severe blood loss, the blood picture with respect to either variable can vary drastically from day to day in the recovery period. At this point, all that can be said is that future work may show a relationship between the lactate content of the blood and the leucocyte count.

The effect of temperature on blood lactate. It may be that the mechanism operating to bring about an increase in blood lactate in the dog when the daily temperature fluctuates around the mean point is of a nervous nature, involving the structures usually acting in temperature regulation. Yamada (1940) has shown that, in rabbits, after bilateral splanchnicotomy there is not obtained the usual hyperlactacidemia following temperature changes. This might indicate a possible involvement of the adrenal medulla, the secretion of which has been shown to influence the blood lactate level (Cori, 1925).

The effect of diurnal variation on blood lactate. Considering, then, the above

demonstrated facts regarding the rhythmic variations of leucocytes in the blood stream and also the blood lactate changes, one may be justified in adopting as a hypothesis the conception that lactate changes in the blood of the resting dog are, in a measure, dependent upon and/or associated with the variations in numbers of leucocytes. This hypothesis must be regarded as provisional until further work of a direct character determines whether or not it can be regarded as valid.

SUMMARY

1. An average value for blood lactate in the resting dog of 10.8 mgm. per cent was found. A total of 234 samples was used.

2. The blood lactate level during recovery from severe, acute hemorrhage of 25 to 32 per cent of the total blood volume in three male and three female dogs was studied by taking daily samples of peripheral blood. Each daily sample was drawn at the same hour, the subjects being in complete rest.

3. After hemorrhage high blood lactate values were found in five dogs at the forty-eighth hour and were still present at the seventy-second hour. One dog varied from the general response in that the blood lactate peak was observed on the twenty-fourth hour.

4. From the third day until the end of the ninth day, when the study ended, the blood lactate curve showed well defined peaks and troughs, indicating a rhythmic variation as compared to the random blood lactate variation of the normal resting subject.

5. No significant difference according to sex was observed.

6. Evidence was given which suggested that the variation in leucocytes may account for some of the phenomena following severe hemorrhage.

7. Blood lactate in dogs did not seem to vary with the day by day fluctuation of the 11 a.m. temperatures.

8. No direct relation was shown between monthly mean blood lactate and monthly mean temperature.

9. A close correlation did exist between monthly mean blood lactate and "temperature mean deviation." By "temperature mean deviation" is meant the dispersion of the daily temperature from the monthly mean temperature.

10. It is suggested that a nervous mechanism may be responsible for the regulation of these variations, since other work has shown, in the rabbit at least, that bilateral splanchnicotomy can check lactic acid production following large temperature changes.

11. There was a well defined diurnal variation in the blood lactate of the resting, normal dog.

12. The twenty-four hour period exhibited two crests. One at mid-day lasted well into the afternoon. The other occurred approximately at mid-night and was of shorter duration than the one at mid-day. It may be, however, of greater magnitude than the one at noon.

13. A third crest was observed in the late afternoon near 6 to 7 p.m. This peak was shorter, more spiked in appearance, and more inconstant in occurrence.

14. Data from the literature describing the diurnal variation of leucocytes are presented showing a similarity between the changes in concentration of leucocytes and blood lactate during the twenty-four hour period.

15. A provisional hypothesis linking the two phenomena is suggested.

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MUCUS, ACID AND WATER SECRETION IN THE STOMACH FOLLOWING THE INJECTION OF PILOCARPINE^{1, 2}

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It is generally believed that pilocarpine, injected subcutaneously, "activates chiefly the production of gastric mucus and enzymes", whereas the secretion of HCl and water are stimulated only secondarily and to a small extent. A critical review of the literature on the gastro-secretory activity of pilocarpine (5), however, reveals that this conclusion is supported by only a fraction of all the evidence reported on this subject since 1875, when jaborandi was first introduced into Europe. Many investigators³ obtained considerable outputs of acid secretion, in some cases comparable with the response to food or to histamine injection. Also, in many instances the content of visible mucin in pilocarpine juice was found to be but little greater than in histamine or post-prandial secretion. The following investigation was undertaken in an effort to resolve the conflict among these many reports, by the use of a different experimental approach to the problem. The question of pepsin secretion was not considered in the present study.

EXPERIMENTAL DETAILS. For the various experiments here described we employed 6 dogs, provided with greater curvature pouches of varying degrees of vagal innervation, i.e., 3 Pavlov, 2 Heidenhain, and 1 of our own type of vagal pouch (8). The mouth of each pouch was provided with a "sphincter", thus making it possible to collect the secretion by either of the following methods: 1, the usual, *continuous collection technique*; 2, the *discontinuous collection or retention technique*, whereby the gastric juice is allowed to accumulate within the small stomach in the absence of the collecting catheter (6). The latter investigation showed that even a small rubber catheter, when held in the pouch throughout an experiment, will induce the secretion of an appreciable amount of visible mucus and consequently some reduction of acidity, though less than is likely to be obtained with a permanent cannula. With the retention technique, however, this mechanical stimulation of mucus is reduced to a minimum (its duration is never greater than half a minute) although the expression of mucus by rubbing of mucosa against mucosa, as a result of increased muscular activity, may occur in the retention experiments as well as in the continuous collection ones.

No experiment was started unless the pouch had been inactive for at least 30 minutes before injection of the stimulus, as indicated by the complete absence of

¹ A preliminary report of this work was presented before the American Society of Biological Chemists in 1938 (9).

² The authors gratefully acknowledge the grants in support of this work received from The Dazian Foundation and from John Wyeth and Brother, Inc.

³ Bibliographic references of historical interest alone are furnished in detail in the aforementioned review and for this reason they are kept to a minimum in the present, experimental report.

free acid. Pilocarpine hydrochloride (hereafter represented by P) solutions, prepared fresh each time, were injected subcutaneously in doses of 1.0, 0.5, 0.05, 0.025 or 0.01 mgm. per kgm. body weight; the first two of these will sometimes be referred to collectively as the upper dosage range, the other three as the lower. Histamine hydrochloride (hereafter represented by H) was administered similarly in a dose of 0.3 mgm. per kgm., which represents about the same number of moles as does the 0.5 mgm. dosage of Pilocarpine HCl. Body weights of the dogs varied from 4.5 to 14 kgm. Records were kept of the frequency and intensity of vomiting and also of intra-pouch bleeding as evidenced by the color of each specimen; an index system of 0, 1, 2 or 3 pluses was found adequate for the comparative purposes of this aspect of the study. Duration of an experiment (total time) was measured from the time of injection (irrespective of the latent period of secretion) until the return of the secretory rate to its basal level. The observations also included the total volume of secretion collected every 15 minutes and the volume of visible mucin which sedimented in the graduated centrifuge tube after being spun for 10 minutes at 2700 R.P.M. Acidities were determined by semi-micro titration and total chlorides by a micro-modification of the Volhard method (10). Neutral chloride was calculated by difference between total chloride and acidity. It should be noted that the phenol red end-point for total acid is not as reliable in the presence of mucin as in its absence, but this is not true for the brom-phenol blue end-point for free acidity. Dry weights, both for dissolved organic solids and for ash, were determined by methods previously described (4).

As a measure of the response of the parietal cells to different procedures or agents, several criteria were available to us: 1, the maximum free and total acidities attained in any single experiment (expressed in milli-normal concentration); 2, the total quantity of acid (calculated as milli-equivalents of HCl) secreted throughout the experiment; and 3, the average acidity throughout the experiment. In order not to prejudice our evaluation of secretory responses, we employed all 3 of these criteria. For the third, a simple arithmetical mean of the titration values for all the specimens appears to possess less validity than a weighted mean which takes cognizance of variations in volume of secretion as well; i.e.,

$$\text{average acidity} = \frac{\sum (\text{acidity} \times \text{volume, for each specimen})}{\sum (\text{volume for each specimen})}$$

Since it is sometimes stated that P stimulates the secretion chiefly of the organic constituents of gastric juice whereas H stimulates HCl and water, we included the total volume of fluid as another measure of secretory activity, and also determined the percentage of soluble organic and inorganic solids in a number of selected specimens. In order to simplify the comparison of data obtained under different experimental conditions, the results are reported in terms of an average for all the experiments in each group performed under essentially the same conditions, rather than in terms of the individual experiments. This statistical procedure was considered valid because the secretory responses to gastric stimu-

lation gave no indication of being characteristic of the individual dogs, whether they possessed a vagotomized pouch of the Heidenhain variety or one of the 2 types of vagal pouch.

RESULTS. *General observations.* The responses to the various doses of P, when only the continuous collection technique was employed, are summarized in table 1; those based on the retention technique are presented in table 4. The

TABLE 1

Mucus, acid, and fluid responses to pilocarpine and histamine; continuous collection experiments

(Data reported as averages for entire groups of comparable experiments)

	STIMULUS					
	Pilocarpine					Hista- mine
	Dosage (mgm./kgm.)					
	1.0	0.5	0.05	0.025	0.01	
1. Number of experiments per group.....	9	9	6	4	4	9
2. Total volume of precipitated mucin per experiment (ml.).....	1.22	1.06	0.25	0.20	0.20	0.09
3. Total duration of secretion per experiment (hours)...	4.00	2.81	1.25	1.19	1.06	1.83
4. Rate of mucin secretion (ml. per quarter hour).....	0.081	0.099	0.053	0.056	0.046	0.014
5. Total quantity of acid (meq. $\times 10^{-3}$).....	2.01	1.77	0.17	0.09	0.03	2.57
Free/Total.....	2.25	1.93	0.19	0.10	0.04	2.63
6. Maximum acidity (mN)....	111	119	66	49	18	143
Free/Total.....	119	126	73	54	25	147
7. Mean acidity (mN).....	84	102	59	44	15	131
Free/Total.....	94	112	65	50	22	135
8. Total volume of fluid per experiment (ml.).....	23.4	17.6	2.7	1.8	1.6	19.9
9. Rate of fluid secretion (ml. per quarter hour).....	1.46	1.57	0.54	0.38	0.38	2.72
10. Blood per experiment (crude index).....	16	8	1	0	0	1
11. Number of vomitings per experiment (crude index)...	4.3	1.1	0	0	0	0

frequency of vomiting which occurred with the two highest doses, and the degree of bleeding and of restlessness, were usually so great as to preclude the use of a dose greater than 1.0 mgm. per kgm. with safety to the animal and the experiment. At the lower dosage levels, there was no evidence of bleeding whatever and vomiting was reduced to a negligible amount. Micturition and defecation were induced in many of these experiments and the mucus content of the feces was frequently very high. Salivation was considerably more profuse than gastric

secretion and it started promptly after injection, whereas the latent period for the pouch secretion was sometimes as long as 15 minutes. The gastric fluid was invariably clear, like the secretion obtained in response to H—except for greater amounts of coagulated mucin.

The duration of gastric secretion, following a single injection of P in the upper dosage range, was considerably longer than for the H experiments, in consequence of a relatively long period of gradually falling acidity with the former stimulus. For purposes of comparison a series of experiments with H were also included. As usually happens with this stimulus, its administration was followed by no vomiting whatever and, but rarely, by slight bleeding of mechanical origin.

The output of insoluble mucin by continuous collection. Let us first consider the response of the mucus cells as measured by the volume of visible mucin obtained after centrifuging—the method used by many previous investigators. The standard dose of H gave an average output of only 0.09 ml. per experiment (line 2, table 1), whereas following the injection of P, the volume of precipitated mucin was considerably greater, irrespective of the dose. The quantity increases with dosage of P, that for 1.0 mgm. being 6 times that for 0.01 mgm./kgm. Since the duration of secretion (line 3) also varies considerably with the dosage of P, the mucus output is obviously correlated with the total time (though not linearly), and the response was therefore studied in terms of mean volume per unit time (line 4). This secretory rate likewise shows a downward trend with decreasing dosage, but the ratio between the extreme values is only about 2:1 instead of 6:1. Similarly, the response to 1.0 mgm. of P per kgm. is 14 times the response to 0.3 mgm. of H in terms of mucin volumes, but in terms of secretory rates their difference is only 6-fold. It must be concluded, therefore, that P, at all the dosage levels here employed, yielded more insoluble mucin than did the control experiments with H, whether the situation was studied in terms of absolute quantities or rates of secretion. Compared with the rate of fluid output (line 8), however, the mucin rate values were extremely small being of the order of 2 to 3 per cent of the former in the lower dosage levels and 0.5 per cent in the higher. It might be expected that the absolute amount of mucin would increase with diminished intensity of stimulation, analogous with the responses to electrical stimulation of the vagi reported by others; but in spite of a 100-fold variation in dosage in these experiments the reverse was actually the case.

Acid and fluid secretion by continuous collection. If the total quantity of HCl secreted in any experiment be taken as a measure of the parietal cell response, we find (line 5) that the highest dosage of P gave on the average only 15 to 20 per cent less acid than did H in its standard dosage. Lower doses of P gave correspondingly smaller quantities of HCl, but even one as low as 0.01 mgm. per kgm. yielded an appreciable amount of acid. The data for total volume of fluid (line 8) and rate of fluid secretion (line 9) also reflect this downward trend with dosage, but although the rates never were as high as that for H, the total volume of fluid obtained with 1.0 mgm. of P actually exceeded that with the standard dose of H by 18 per cent—contrary to what might be expected from a large part of the literature. Total duration of an experiment (line 3) also shows a positive corre-

lation with dosage, and the P experiments with the two higher doses lasted very much longer than the H experiments. Analysis of the acid response in terms of the maximum acidity attained in each experiment (line 6), or the average acidity throughout the experiment (line 7), yields a slightly, though not materially, different picture. By both of these criteria, the highest response was obtained at the 0.5 mgm. dosage level, rather than 1.0 mgm., the difference between them being 7 to 8 mN (6 per cent) in terms of maximum acidity and 18 mN (16 per cent) in terms of mean acidities. These differences are of uncertain statistical significance, but if they have any validity at all they can be only a secondary consequence of several other factors operating in combination—like the rates of mucus and fluid secretion and the amount of vomiting. Comparing the response to 0.5 mgm. of P with the corresponding response to 0.3 mgm. of H, we find that both these acidity measures are lower in the P group; in terms of maximum total acidity the difference is 14 to 16 per cent, and in terms of average total acidity it is 17 to 20 per cent. However, these differences, as well as the analogous difference in terms of total quantity of acid, are all sufficiently small so as to indicate that P in these larger doses may still be considered a good stimulus to HCl as well as to fluid secretion, even as compared with histamine. Although the rate of secretion of fluid (line 9) is materially faster in the H experiments than in any of those with P, irrespective of dosage, doses of H somewhat less than 0.3 mgm. per kgm. would undoubtedly have given comparable results even in this respect.

Chloride secretion by continuous collection. Estimations of total and neutral chloride concentrations were performed whenever the specimens were large enough to permit of such determinations parallel with acidity titrations. Total chloride values are generally high with but small variations during any one experiment; values for neutral chloride tend to be correlated inversely with the acidity, as we have previously shown to be the case for H secretion. This relation is indicated by the group averages of table 2, and it is amply demonstrated by analysis of the data for individual experiments. Hence, P is not essentially different from H in respect to chloride concentrations, and the variations which are observed are only such as may be expected from admixture of mucus and parietal cell secretion in different amounts.

Total dissolved solids by continuous collection. The volume of insoluble mucin represents only one aspect of mucus secretion, and crudely at that, since it disregards the mucin which remains in solution. In order to check on this soluble factor, we determined the concentration of dissolved solids in a number of specimens of P secretion; for purposes of comparison, a like number of determinations were made on H juice (table 3). The individual specimens were chosen with a view to their yielding 2 groups of data with approximately the same mean acidities, and also to their being large enough for analysis. In general, volume of specimen (actually, rate of secretion when the time factor is held constant) is correlated positively with the acidity, for P as well as for H and food; in accordance with this the P specimens of table 3 possess a mean acidity considerably higher than any of the corresponding values of table 1 (line 7), and more nearly

equal to the maximum value (line 6) for this same dosage level (0.5 mgm. per kgm.). The 2 groups of data for ash content are in reasonably good agreement with each other, as might be expected from their identical acidities, but the concentration of organic solids in the P secretion is almost twice that in the H secretion. The actual concentration ratio of P to H is 1.8, whereas for precipitated mucin the analogous ratio is about 13 (data from table 1, line 2, adjusted

TABLE 2

Chloride and acidity data; continuous collection experiments

STIMULUS AND DOSE	EXPERIMENT NUMBER	SPECIMENS TITRATED	MEAN Cl CONCENTRATION		MEAN ACIDITY	
			Total	Neutral	Free	Total
			<i>mN</i>	<i>mN</i>	<i>mN</i>	<i>mN</i>
Pilocarpine 1.0 mgm./kgm.	J-26a	3	156	73	74	82
	J-28	11	159	73	78	86
	J-33	7	157	66	74	85
	J-35	14	147	37	95	107
	J-36	14	160	42	108	116
	J-38	10	155	63	78	85
	J-48	6	151	51	80	95
	J-49	10	152	41	93	109
	J-54	4	157	70	73	79
	Mean	79	155	57	84	94
Pilocarpine 0.5 mgm./kgm.	J-2	7	150	57	68	93
	J-7	3	157	56	90	101
	J-9	7	158	27	120	131
	J-14	7	156	28	120	126
	J-25	12	154	35	104	114
	J-56	7	151	54	84	90
	Mean	43	154	43	98	109
Histamine 0.3 mgm./kgm.	J-12	5	160	14	137	142
	J-13	2	165	28	128	131
	J-24	8	161	13	142	144
	J-30	3	159	20	135	138
	J-32	5	160	38	118	122
	J-34	7	158	28	127	130
	Mean	30	160	24	131	135

for the total volume of fluid per experiment, in line 8). Hence the difference between these two stimuli as regards their output of dissolved organic matter is less than for insoluble mucin, but it is still sufficiently great so that there can be no question of its validity.

Visible mucus by the retention technique. From the foregoing data, it is evident that the P experiments yielded more mucus secretion than did the H experiments,

irrespective of the dosage level of the former, but it does not necessarily follow that the mucus cells were stimulated directly by the pilocarpine, acting at the neuro-glandular junction. From our previous experience with histamine alone and with ingested food we know that some, at least, of this mucus may have been evoked mechanically—in part, by rubbing of the mucosal surface against the catheter, and in part, by being squeezed out of the cells as a result of the increased activity of the gastric musculature. Any pharmacological procedure for controlling these mechanical processes entails the risk of simultaneous influence on the direct stimulation of the mucus cells, if any such exists. The rubbing effect, however, can be eliminated in great measure without this risk by use of the

TABLE 3

Dissolved organic solids and ash of pilocarpine and histamine secretions (filtered); continuous collection experiments

SPECIMEN NUMBER	STIMULUS	ACIDITY		ORGANIC SOLIDS	ASH
		Free	Total		
	mgm./kgm.	mN	mN	mgm./100 ml.)	mgm./100 ml.
23-1	Pilocarpine	127	132	0.48	0.06
2	0.5	122	129	0.41	0.08
3	mgm./kgm.	108	115	0.44	0.18
4		123	130	0.28	0.21
5		148	152	0.41	0.29
6		116	123	0.22	0.16
7		137	142	0.32	0.21
Mean		126	132	0.37	0.17
23-8	Histamine	102	106	0.29	0.34
9	0.3	147	150	0.06	0.13
10	mgm./kgm.	99	107	0.44	0.33
11		140	143	0.20	0.14
12		121	126	0.22	0.26
13		143	147	0.14	0.13
14		145	149	0.10	0.12
Mean		128	133	0.21	0.21

retention technique for collecting the secretion. Accordingly we performed 2 series of experiments by this method, using P in doses of 1.0 and 0.5 mgm. per kgm. respectively. The results are compared in table 4 with corresponding data from the continuous collection experiments.

Although the durations of the 2 groups of retention experiments (line 3) were not significantly different, on the average, from those by the other procedure, the total volume of precipitated mucus per experiment (line 2) and the rate of mucus secretion (line 4) are both distinctly less in the retention group. In terms of total volume of precipitated mucus, the differences are 47 and 58 per cent of the median values for the 1.0 and 0.5 mgm. dosages respectively; in terms of secretory rate,

they are 58 and 98 per cent. Because of the crude method of measuring mucus output, and the fact that the frequencies (number of experiments per group) are never greater than 9, these differences are not subject to reliable statistical evaluation. Their validity, however, is confirmed by the data on maximum and mean acidities—both free and total (lines 6 and 7). For both of these criteria and for both the dosages of P, the retention data are invariably greater than the corresponding continuous collection data; the greatest of these differences is 25 per cent of the median, the least is 10 per cent. The differences in terms of mean acidity are greater than in terms of maximum acidity, and since the latter data

TABLE 4

Comparison of secretory responses to pilocarpine with different methods of collecting gastric juice (data reported as averages for entire groups of comparable experiments)

	DOSE OF PILOCARPINE			
	1.0 mgm./kgm.		0.5 mgm./kgm.	
	Method of collection			
	Continuous	Retention	Continuous	Retention
1. Number of experiments per group.....	9	9	9	8
2. Total volume of precipitated mucin per experiment (ml.).....	1.22	0.75	1.06	0.47
3. Total duration of secretion per experiment (hours).....	4.00	4.03	2.81	3.06
4. Rate of mucin secretion (ml. per quarter hour).....	0.081	0.047	0.099	0.034
5. Total quantity of acid (meq. $\times 10^{-3}$).....	<u>2.01</u>	<u>2.94</u>	<u>1.77</u>	<u>1.74</u>
Free/Total.....	2.25	3.36	1.93	1.88
6. Maximum acidity (mN).....	<u>111</u>	<u>125</u>	<u>119</u>	<u>133</u>
Free/Total.....	119	140	126	140
7. Mean acidity (mN).....	<u>84</u>	<u>105</u>	<u>102</u>	<u>122</u>
Free/Total.....	94	120	112	130
8. Total volume of fluid per experiment (ml.)...	23.4	27.5	17.6	15.5
9. Rate of fluid secretion (ml. per quarter hour)...	5.85	6.82	6.26	5.07
10. Blood per experiment (crude index).....	16	15	8	7
11. Number of vomitings per experiment (crude index).....	4.3	3.2	1.1	0.3

are based on fewer than 25 values, they also are not subject to statistical analysis with any degree of confidence. The mean acidities, on the contrary, are calculated for frequencies which vary from 98 to 145 specimens per group of experiments. For such frequencies, the usual significance test for the difference of 2 means is acceptable, and its application indicates that all 4 mean acidity differences are significant at the 5 per cent level of probability. Comparative experiments of this kind were not performed with the lower dosages because of the very small quantities of secretion which these yield and the consequent increase in variability of the observations.

From these findings it may be concluded that, for a given dose of P, the reten-

tion technique yields both higher acidity values and lower mucin values than does the continuous collection procedure,⁴ the data being in accord with the idea that these higher acidities result from diminished secretion of, and neutralization by, mucus. It is interesting that, irrespective of the dosage, the amount of bleeding and the frequency of vomiting appeared to be less in the retention experiments than in the others, even though the quantitative validity of such differences cannot be established. Apart from the influence of these two factors, however, the greater lowering of acidities (from the characteristic value for the parietal fluid, about 167 mN) in the continuous collection experiments must be ascribed to the mechanical stimulation of the mucus cells by the collection catheter. Elimination of this stimulus results in a diminished output (or secretory rate) of mucus and consequent elevation of acidity. Were it possible also to inhibit specifically the motor effects of the P, these differences between the two groups of experiments involving the same dosage of P but different collection techniques would be even greater, i.e., the mucin contents of the retention experiments would be less and the acidities higher than those actually observed.

DISCUSSION. We have found that P, in doses of 0.5 and 1.0 mgm. per kgm., yields concentrations and amounts of HCl, which, though less than those given by 0.3 mgm. of H per kgm., are of the same order of magnitude. No experiment was performed unless there was a complete absence of acid secretion prior to injection of the stimulus, and at no time were 2 stimuli superimposed on each other. Previous investigators failed to take the first of these precautions, and several actually report the simultaneous administration of P with H or food (17). Hence the present study reflects solely the stimulating power of P. As for the secretion of water, the H data are actually intermediate between those for P at the 2 upper dosage levels, but the smaller amounts of both HCl and water, obtained with lower doses of P, in no way affect the conclusion that, *under suitable conditions, P stimulates the flow of gastric HCl and fluid in amounts comparable with H*. The responses from differently innervated pouches were essentially the same, consistent with the observations of others. The identity of the acid/chloride relations for P and H secretion affords further evidence of the essential similarity of these two types of secretion.

Besides the dosage of P, two other factors are responsible for the low concentration of HCl relative to the H results. One is the lower rate of acid secretion, which ordinarily is correlated positively with acid concentration (3, 13), but this was offset by the greater duration of response to a single injection, at higher dosage. As a result the total quantities of acid in the P and H groups were in better agreement than were the corresponding concentration data. The second factor is the greater rate of mucus secretion under the influence of P. Our data (7) indicate that the buffer capacity of this secretion may be even greater than has hitherto been reported; hence though the quantitative response of the parietal cells

⁴ Individual acidities of the continuous collection experiments with P never quite attained the highest values of the H experiments (149/152 mN). The retention experiments, however, actually exceeded these values; with 1.0 mgm. of P the highest individual acidity was 155/160 mN, and with 0.5 mgm. of P it was practically identical 156/160 mN).

may be the same for a pair of P and H experiments, the greater activity of the mucus cells in one of this pair must lower the acidity throughout that experiment. Had we not taken pains to minimize mucosal irritation and trauma, the observed acidities would have been even lower for any set of experimental conditions. These factors, and the several methods of analysis applied to our data on acid output, serve to explain the conflicting results reported by previous investigators of these phenomena.

Concerning mucus output the picture is more involved. P clearly induces greater activity of the mucus cells than does H, as shown by the relative rates of secretion of visible mucin and the data on dissolved organic solids, even for the lower dosages of P. Had the dry weight determinations been performed on specimens of higher acidity, the values for organic solids would have been even lower—because of the negative correlation between the concentrations of dissolved solids and HCl (4)—but the difference between the 2 stimuli would probably have persisted in some measure. Hence P administration undoubtedly increases the output of mucin but there must be considerable doubt about the mechanism of stimulation. In general, P exercises a vagomimetic function in the stomach but histological evidence for the parasympathetic innervation of the mucus cells is lacking. Otherwise one might expect the output of mucus to be considerably greater after P than by “spontaneous” secretion, but this proves not to be so.⁵ Even the volume of mucin relative to that of fluid in the P experiments is small, the ratios being 0.052, 0.060, 0.093, 0.111 and 0.125, for descending dosages.

It has been reported by Uschakow (15) and Vineberg (16) that electrical stimulation of the vagi gives acid secretion if the induced current be strong, but mucus (frequently followed by acid) if it be weak. The data from lines 2 and 4 of table 1 are not at all consistent with this differential reaction because the values decrease with decreasing dosage; also, though the mucin:fluid ratios rise with diminishing dosage, this results from decreasing fluid volumes rather than increasing mucin volumes. On the other hand, the experiments of table 4 demonstrate that a large portion of the mucus output is the result of mechanical stimulation of the mucosa by the catheter. Observations on humans (2, 11) and on gastric fistula dogs (1) have shown that increased motility, even of hunger contractions, increases the “spontaneous” secretion of mucus. Hence, another

⁵ We have compared the rate of mucin secretion for the various groups of P experiments (table 1, line 4) with similar unpublished data on the rate of “spontaneous” secretion of mucin, measured by continuous collection 18 to 24 hours following the last meal. With P, the rates (in ml. per quarter hour) vary from 0.046 to 0.099; “spontaneous” mucus secretion in the absence of free acid (pH 6.0) has a mean rate of 0.10 (20 expts.), and when accompanied by acid secretion (pH 3.0) of 0.03 (17 expts.). The non-acid values represent viscous mucus, whereas the acid values are for insoluble mucin. Had the non-acid “spontaneous” specimens been acidified and centrifuged, their values in terms of precipitated mucin would probably have averaged between 0.05 and 0.07 ml. per quarter hour. If the resting secretion of acid is actually psychic, and therefore vagal, we might expect that the mucus cells would also be stimulated vagally in such experiments and that the acid specimens would contain more mucin than the non-acid ones. The fact that the reverse is true casts further doubt on the parasympathetic innervation of the columnar epithelium.

large fraction of the mucus output after P must be ascribed to such mechanical expression; elimination of the motor component of the response to P without interfering with the secretory component would probably reduce the rate of mucus secretion to an exceedingly low value. H induces no motor activity (12) and therefore only a negligible amount of mucus secretion (6, 14). Whether the output of P mucus can ever be lowered to the level of the H experiments can be established conclusively only by the selective elimination of these secondary mechanisms of stimulation; in the light of the reduction already effected by use of the retention technique of collecting secretion, it seems not at all improbable. Hence it may be concluded that (1) *a major part of the mucus secreted in the P experiments is stimulated mechanically, in several ways, and (2) it is doubtful whether any of this secretory activity is induced by direct action of the drug at the neuro-glandular junction.*

SUMMARY

1. Pilocarpine, when injected subcutaneously into stomach pouch dogs in adequate dosage, stimulated the flow of gastric HCl and fluid in amounts comparable with those evoked by histamine. The secretory-time curves were generally irregular but were characterized by relatively long latent periods and total durations of response to a single injection.

2. Administration of pilocarpine was accompanied also by the secretion of gastric mucus in considerable quantity, especially when the continuous collection technique (with a catheter in situ throughout the experiment) was employed. The rate of secretion, however, did not differ materially from that of the "spontaneous" secretion of mucus in acid-free pouches. Furthermore, a major part of this mucus-secretory activity was eliminated when the retention technique was used for collecting the pouch contents (in the absence of a solid collecting device), thus demonstrating the mechanical (in contradistinction to direct pharmacological) origin of the mucus which is ordinarily evoked by this stimulus. Evidence from other laboratories indicates also that a considerable part of the mucus is squeezed out from the surface epithelial cells by the muscular activity induced by the pilocarpine, even in the absence of rubbing by a foreign body. Hence it is questionable whether any of the mucus-secretory activity is induced by direct action of the pilocarpine at the neuro-glandular junction.

3. In agreement with previous observations, high dosages of the drug (0.5-1.0 mgm. per kgm.) induced restlessness, salivation, mucosal bleeding, vomiting and defecation. Oozing of blood in the pouch occurred to a greater extent in the continuous collection experiments than in those performed by the retention technique.

4. This investigation gives further evidence of the importance of the retention technique for certain kinds of quantitative investigations of gastric secretion.

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A COMPARISON OF THE CONFIGURATION IN THE ELECTROCARDIOGRAM OF ENDOCARDIAL AND EPICARDIAL EXTRASYSTOLES^{1,2}

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Lewis considered the theory of "limited potential differences" as an explanation of the electrocardiogram to be established by the observation (1) that the initial deflection of extrasystoles elicited from the endocardium of the right ventricle in the dog was downward, while that from the overlying epicardial surface was upward. This observation was explained by assuming that in the first instance a limited region of potential difference was created with the negative poles oriented toward the inside of the heart, while in the second instance the dipoles were reversed because the primary direction of propagation was from without inward.

The results of experiments on external stimulation of the right and left ventricles, summarized in other papers (2, 3), suggest that there is another interpretation of the phenomenon described by Lewis. Extrasystoles elicited from the right ventricle do show downward initial deflections in certain leads when the surface is stimulated at almost any point except in an area around its center. (By the center of a ventricle is meant that region of its surface at its lateral border which is equidistant from all points along the septum.) These downward deflections have already been shown to have their origin in excitation of the left ventricle (2).

Conversely, it has been shown that extrasystoles elicited from the left ventricle show downward initial deflections in all leads only when the point of stimulation is in a restricted area at the center of that ventricle. When other points are stimulated the resulting initial deflection is upward in some leads, and this deflection has been shown to arise from excitation of the right ventricle (3).

The suggestion arising from these observations is that downward initial deflections produced by stimulation of the endocardial surface of the right ventricle might also have the same origin as the downward deflections induced by stimulation of the external surface, namely, excitation of the opposite ventricle. Other experiments on this topic throw no certain light on the subject (4, 5).

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² This paper was submitted with the original electrocardiograms as evidence of the results obtained. As a measure to conserve space and material the authors were requested to substitute for them a single figure (fig. 1) in which the results are illustrated by means of line drawings. These drawings follow as closely as possible measurements taken from actual electrocardiograms.

METHODS. Thirteen dogs were employed, prepared as previously described (6). An electrode holder was made to hold in juxtaposition two dipolar electrodes. One limb was inserted into the ventricle through the auricle and the other limb was applied directly opposite it on the external surface of the heart. By means of a thyatron stimulator, connected to the electrodes through a commutator, extrasystoles could be elicited from points directly opposite each other on the endocardium or the epicardium. Both the right and the left ventricle were explored. Electrocardiograms were taken from leads I and III, the lungs being fully expanded and the edges of the skin and subjacent muscles approximated with clips.

RESULTS. *A. Regions in which initial deflections of endocardial and epicardial extrasystoles were in same direction.* The first result of these experiments was the finding that in most regions of the heart stimulation of the endocardium and the overlying epicardium yielded complexes in which the initial deflections were not oppositely directed as Lewis found, but were the same. These complexes varied only in that the QRS interval of internally excited extrasystoles from the left ventricle was less than QRS in externally elicited extrasystoles from the same ventricle.

When the center of the right ventricle was stimulated the initial deflection was, as usual (7), upward in leads I and III when the stimulus was externally applied (fig. 1, 1 out). It was also upward when the endocardium was stimulated (fig. 1, 1 in). When the center of the left ventricle was stimulated, extrasystoles from the outside showed downward initial deflections in leads I and III (fig. 1, 3 out) (7). Extrasystoles elicited from the endocardium showed also a downward initial deflection (fig. 1, 3 in).

When points along the anterior septum were stimulated externally, lead I showed a downward initial deflection, while lead III showed an upward initial deflection (7). When subjacent points were stimulated on the interior of the heart, either in the right ventricle or in the left ventricle, the initial deflections of the resulting complexes were similarly downward in lead I and upward in lead III. The direction of the initial deflections was therefore the same, whether the endocardium or the epicardium was stimulated (fig. 1, 2 in and out).

Complexes elicited by stimulating the endocardium of the right ventricle and the overlying epicardium at the posterior septum were also identical with regard to the direction of initial deflections (fig. 1, 4 out and in). Because of technical difficulties, the endocardium of the left ventricle at the posterior septum was not stimulated.

Epicardial and subjacent endocardial stimulation of numerous points over the entire anterior right ventricle gave extrasystoles in which the initial complexes were also in the same direction. The configuration of extrasystoles elicited by stimulation of the epicardium in this region has already been described (2). In lead III there is no change as the electrode is moved laterally from the anterior septum. In lead I there is a change from the downwardly directed initial complex characteristic of an anterior septal extrasystole to the extrasystole produced by stimulation of the center of the right ventricle, in which the initial complex is directed upward. In this transition the initial downward deflection becomes smaller and an upward deflection appears following it, which increases in ampli-

tude as the point of stimulation approaches the center. The complex thus appears to have a Q of diminishing amplitude and an R wave of increasing amplitude. The same sequence was found when subjacent points on the endocardium were stimulated, and no points were found at which the initial deflections of epicardial and endocardial extrasystoles were oppositely directed. The initial deflections in lead III were also similarly directed in endocardial and epicardial extrasystoles, although of course in this lead there was no change in configuration with change in the position of the electrode (fig. 1, c and d, out and in).

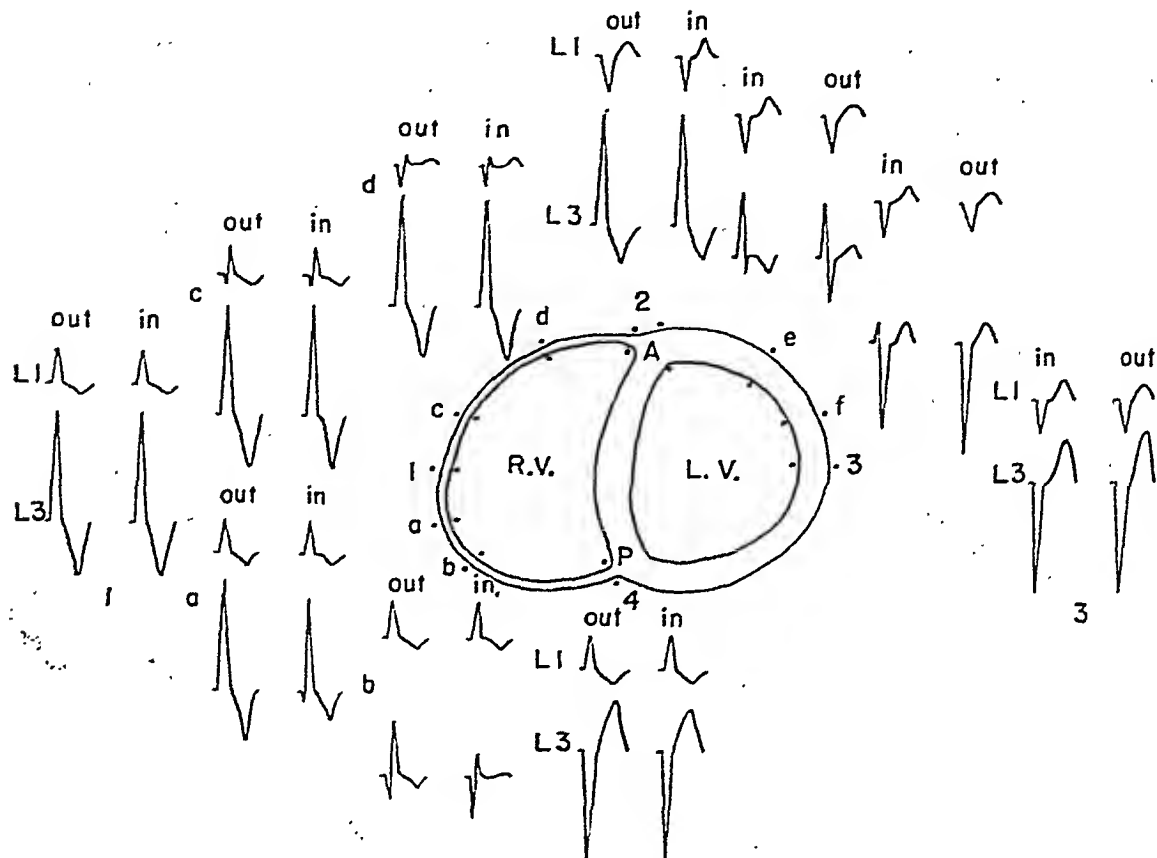


Fig. 1. A semischematic summary of the configuration in the electrocardiogram of extrasystoles elicited from epicardial and endocardial points. See text for explanation. The relations here depicted hold over a broad belt reaching from the base nearly to the apex.

B. Regions from which extrasystoles with divergent initial complexes could be obtained. (i) Anterior left ventricle. In four experiments the anterior left ventricle was thoroughly explored. The paired electrodes were moved progressively from the center of the left ventricle anteriorly toward the septum. At the center of the left ventricle initial complexes of both endocardial and epicardial extrasystoles were directed downward in both leads I and III (fig. 1, 3). As the electrode was moved toward the anterior septum, and at the septum, lead I remained unchanged both in endocardial and epicardial extrasystoles, which showed an initial downward deflection (fig. 1, e and f, lead I). In lead III extrasystoles elicited by epicardial stimulation of points between the center of the

left ventricle and the anterior septum showed the development of a small preliminary upward deflection (an R wave) which increased in amplitude as the downward deflection (S) decreased, until at the anterior septum only an R wave could be found (3). The extrasystoles elicited from subjacent points on the endocardium showed a similar transition in the development of an R wave. There was, however, one significant difference, namely, that the first appearance of an R wave occurred nearer the center than in the case of epicardial extrasystoles. It was therefore possible to find points in the anterolateral region of the left ventricle in which, in lead III, an R wave was present in the endocardial extrasystole, while there was none in the epicardial extrasystole (fig. 1, f, lead III). The area from which such divergent complexes could be obtained was considerably greater than that on the right ventricle. These complexes form a striking counterpart to the figure published by Lewis for the right ventricle.

Having found, in earlier experiments, that similar upward initial deflections in extrasystoles derived from epicardial stimulation of the left ventricle are produced by excitation of the right ventricle (3), and noting that stimulation of the inside and outside varied only in that the area yielding purely downward complexes was more restricted in the endocardium, it was postulated that the upward deflections of these extrasystoles were also due to excitation of the right ventricle. The anterior right ventricle was accordingly cooled by means of the thermal chamber previously employed (3) (fig. 2, 1 B) while extrasystoles were being elicited which showed in controls such upward initial complexes. The R wave in the normal complexes was reduced in magnitude and the S wave increased. Similarly the R wave of extrasystoles excited by stimulation of the endocardium was reduced greatly, while the S wave was increased (fig. 2, 1 B). Figure 2, 1 C shows the return to normal of both the normal complex and the extrasystole after cooling was discontinued and the heart was allowed to regain its previous temperature.

(ii) *Posterior right ventricle.* In three experiments it was possible to reproduce exactly the configurations described by Lewis for the right ventricle. Figure 1, 4, b, a, 1 shows the changing pattern in lead III of extrasystoles elicited from the epicardium (out) and the endocardium (in) at four points progressing from the posterior septum to the center of the right ventricle. In both "out" and "in" is shown a progression from a complex with downward initial deflection to a complex in which the initial deflection is upward. In both epicardial and endocardial extrasystoles this progression includes complexes having Q waves. The only difference is that the area in the endocardium from which purely upward initial deflections were obtained was more restricted than the area on the epicardium which yielded similar complexes, so that a region exists (fig. 1, a) where in lead III a small Q is present in the endocardial but not in the epicardial extrasystole. In lead I all these extrasystoles remained the same, showing only an upright initial complex or R wave, and no differences were found in the direction of epicardial and endocardial extrasystoles.

It has already been demonstrated that Q waves seen in lead III of extrasystoles elicited by stimulating the epicardium of the posterior right ventricle have their

origin in excitation of the posterior surface of the left ventricle (2). To test whether the similar Q waves in extrasystoles derived from the endocardium in the same regions have the same origin, the posterior left ventricle was heated and cooled while extrasystoles having Q waves were elicited by stimulation of the endocardium in an appropriate region of the posterior right ventricle. The virtual disappearance of the Q wave of such extrasystoles when the left ventricle was cooled (fig. 2, 2 B) and its return when the left ventricle was subsequently

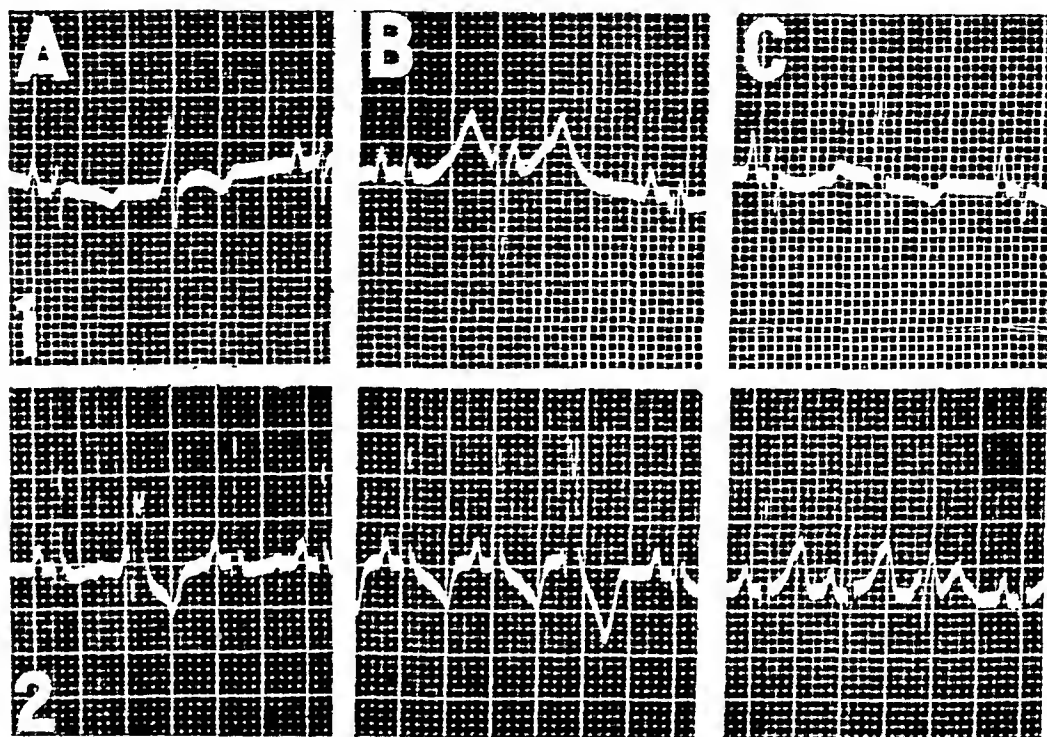


Fig. 2. 1 A, B, C. Nov. 19, 1942. 6.0 kgm. dog. Lead III. 1 A. Shows an extrasystole elicited by stimulating the left ventricular endocardium at a point near the anterior septum. 1 B. Shows great reduction in amplitude of R of the extrasystole by cooling the anterior surface of the right ventricle. 1 C. Shows return of R in the extrasystole when the right anterior ventricular surface returns to the normal temperature. 2 A, B, C. Nov. 24, 1942. 9.5 kgm. dog. Lead III. 2 A. Shows a Q in an extrasystole elicited by stimulating the right ventricular endocardium at a point midway between the lateral margin and the posterior septum. 2 B. Elimination of Q of extrasystole by cooling the posterior surface of the left ventricle. 2 C. Return of Q in the extrasystole by warming the surface of the posterior left ventricle.

warmed (fig. 2, 2 C) indicate that the Q wave of endocardial extrasystoles originates in the left ventricle just as does the Q wave of epicardial extrasystoles.

DISCUSSION. In these experiments only two regions of the ventricles were found in which there is divergence in the direction of the initial deflection of extrasystoles elicited from the endocardium and overlying epicardium. These are areas in the posterior part of the right ventricle near the lateral margin of the heart, and in the anterior portion of the left ventricle, also near the lateral border. Here there is divergence only in lead III, and conformity in lead I. In all other regions of the heart studied no divergence was found in the direction of the initial

deflections of extrasystoles from endocardial and overlying epicardial points in either lead I or lead III.

The divergence in right ventricular extrasystoles consists in the appearance of a small Q wave in endocardial extrasystoles while epicardial extrasystoles still show only an R wave. This appearance of a Q wave in endocardial extrasystoles is part of a transition in lead III from complexes elicited at the lateral margin, which show only an R wave, to complexes elicited at the posterior septal region, in which R has been completely replaced by a Q wave. This transition is the same as that undergone by epicardial extrasystoles, the only difference being that a Q wave appears in epicardial extrasystoles at points a few millimeters nearer the posterior septum than those in the endocardium which first show Q waves. Stated in another way, the region in the center of the right ventricle which on stimulation gives exclusively upright initial complexes is more restricted in the endocardium than on the epicardium.

The region at the center of the left ventricle which on stimulation gives exclusively downward initial complexes is also less extensive in the endocardium than on the epicardium. The transition from a purely downward complex in lead III to a purely upward complex in that lead, which takes place as the point of stimulation is moved from the center of the left ventricle to the anterior septum, therefore began nearer the center in endocardial extrasystoles. There is thus a region near the anterior left lateral margin in which the endocardial extrasystole shows a small R while the initial complex of the epicardial extrasystole is still exclusively downward.

The appearance and progressive increase in amplitude of a Q wave as points of stimulation on the epicardium of the right ventricle approached the septum suggested that the Q wave was derived from the excitation of the left ventricle. This concept of the origin of Q was proved by the readiness with which this wave could be increased or decreased or abolished by appropriate treatment of the left ventricle (2). In the present experiments the similarity of progression of the Q wave in endocardial extrasystoles from the right ventricle suggested that these Q waves also had their origin in the left ventricle. The fact that heating and cooling the left ventricle caused important modifications in the Q wave of such endocardial extrasystoles confirms this view. These experiments offer no support to the view that such Q waves arise from conduction of an impulse from endocardium to overlying epicardium in the right ventricle.

In a similar way it was demonstrated that the appearance and progressive increase in amplitude of an R wave in lead III, when points on the endocardium of the left ventricle were stimulated, was due to the progressively earlier activation of the right ventricle. Heating and cooling of the right ventricle modified the R wave of endocardial left ventricular extrasystoles just as they modified epicardial extrasystoles (3). Here again results are inconsistent with an explanation of the R wave on the basis of its origin in the left ventricle in an impulse travelling from endocardium to epicardium.

It was observed frequently that the QRS interval of left endocardial ventricular extrasystoles was definitely shorter than that of corresponding epicardial

extrasystoles. This indicates that conduction to the opposite ventricle takes place more rapidly when the endocardium is stimulated than when the extrasystole arises in the epicardium. If this be so, it can be understood why points were found on the endocardium near the lateral border of the left ventricle from which extrasystoles were obtained showing small R waves, while extrasystoles from overlying points on the epicardium do not show R waves. Divergence in the direction of the initial deflection of right ventricular extrasystoles from endocardium and epicardium must also arise from a slightly more rapid spread of excitation to the left ventricle when the endocardium of the right ventricle is stimulated than when an overlying epicardial point is the locus of stimulation.

It is to be remembered that in Lewis' experiments standard indirect leads were not employed, but that leads were placed on opposite sides of the chest, in line with the electrodes, to create optimum conditions for recording a limited potential arising in the endocardium. The situation is somewhat different in the present experiments. Lack of conformity in initial deflections of epicardial and endocardial extrasystoles was not found in regions which are lined up with the external leads. Thus lead III is known to be in line with the centers of the anterior right ventricle and the posterior left ventricle (8). The areas showing divergence in this lead were, however, found in the posterolateral region of the right ventricle and the anterolateral region of the left ventricle. These latter regions are in line with lead I, which failed to show any divergence between endocardial and epicardial extrasystoles.

SUMMARY

1. The configuration, in leads I and III of the electrocardiogram, of ventricular extrasystoles elicited by stimulation of points on the endocardium and the immediately opposite epicardium has been studied.

2. The direction of initial deflections of endocardial and epicardial extrasystoles was the same in both leads I and III over most of the surface of the heart. These regions included (1) the area over the septum, both anterior and posterior; (2) an area on the lateral margin of each ventricle, which is referred to as the center of the ventricle because it is equidistant from the septal margins, and (3) the entire anterior surface of the right ventricle.

3. The direction of initial deflections in lead I of the epicardial and endocardial extrasystoles was also found to be the same over the entire posterior portion of the right ventricle and the anterior part of the left ventricle.

4. The direction of initial deflections in lead III of epicardial and endocardial extrasystoles was the same over much of the posterior portion of the right ventricle and the anterior part of the left ventricle.

5. Two areas were found, of the anterior left ventricle toward the left lateral border and the posterior right ventricle toward the right lateral border, where the initial deflections of endocardial extrasystoles were opposite to those of extrasystoles from immediately overlying epicardial points.

6. These oppositely directed initial complexes of extrasystoles elicited from

such regions of the endocardium were shown to arise, as do the similar complexes from stimulation of near-by epicardial points, from excitation of the opposite ventricle.

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A RENAL REABSORPTIVE MECHANISM IN THE DOG COMMON TO GLYCIN AND CREATINE

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The development of an extensive glomerular filtering bed in the mammalian kidney has necessitated the parallel development of a similarly extensive tubular reabsorptive surface. The salvaging of water, salts, glucose, amino acids and other constituents of value from the glomerular filtrate constitutes a problem of homeostasis fully as important as that of excretion of waste materials. Quantitatively, reabsorptive processes outweigh purely excretory ones.

The presence of amino acids in the circulating plasma in appreciable concentrations and their almost complete absence from the urine under normal conditions point to an efficient renal mechanism for the reabsorption of these substances. Studies that have been made of excretion following administration of amino acids have not been such as to contribute to our knowledge of the kinetics of the reabsorptive system (Kirk, 1936; Doty, 1941). The importance of amino acids in the general economy of the body lends interest to such a study, while recent advances in therapy involving the parenteral administration of amino acids add an element of practical importance (Elman, 1937).

Experiments designed to outline the characteristics of the reabsorptive system in the dog for the amino acid glycine are presented in this paper. It has been found that glycine is reabsorbed from the glomerular filtrate by a stable active chemical transport mechanism which exhibits, as does the glucose reabsorptive system (Shannon and Fisher, 1938) limitation of transfer capacity. Creatine, which may be considered as a substituted glycine, is reabsorbed by the same mechanism. Hence, when either or both of these substances are presented to the renal tubules in increased amounts, they compete for the common reabsorptive system.

EXPERIMENTAL PROCEDURE. Observations have been made on four well trained female mongrel dogs. During the experiments they were loosely restrained on a comfortable animal board. Urine collections were made with the use of an indwelling catheter. Collection periods were 10 minutes in length, and if the urine flow amounted to less than 10 cc. per minute, the bladder was washed out and the washings added to the original urine. Since peripheral tissues may absorb large amounts of amino acids (Van Slyke and Meyer, 1913) leading to significant arterio-venous differences, all analyses were performed on arterial plasma. Arterial blood sampling at the exact mid-point of each urine collection period was greatly facilitated by the use of an indwelling femoral arterial needle fitted with a tight stylet. Constant creatinine and varying amino nitrogen concentrations in the plasma were obtained by infusions administered through the saphenous vein by means of a motor driven pump. Water, in amounts of 50 cc. per kgm., was administered per os 60 minutes prior to the start of the experiment.

Observations have been made with both rising and falling plasma concentrations of amino nitrogen. During any given period of observation, however, change in concentration has been restricted. This has been accomplished by administering infusions of various concentrations at constant rate for a period of 20 minutes prior to the start of experimental periods to establish approximate equilibrium. In those experiments in which high plasma levels were attained initially, an appropriate priming dose was given before starting the infusion. The slow rate of change of plasma concentration coupled with uniformly high rates of urine flow render dead space errors negligible.

Chemical methods. For amino acid determination plasma and diluted urines were treated with dialyzed, purified urease and a 1:6 picric acid filtrate was prepared. The ninhydrin gasometric determination of Van Slyke, Dillon, MacFadyen and Hamilton (1941) as modified by Hamilton (1942) was applied to the urea-free filtrate. Creatinine, creatine and glucose analyses were performed on iron filtrates of plasma (Steiner, Urban and West, 1932) and on diluted urines. Creatinine analyses were performed by the Folin and Wu (1919) method and creatine by the acid hydrolysis method as modified by Pitts (1934). The colors were read on an Evelyn colorimeter exactly 10 minutes after the addition of alkaline picrate to each tube. Glucose was determined by the Folin (1929) method as modified by Shannon, Farber and Troast (1941). Para-amino-hippuric acid was determined on cadmium filtrates (Fujita and Iwatake, 1931) by the method of Finkelstein, Aliminosa and Smith (1941). Urine pH measurements were made in several experiments using a glass electrode without precaution to prevent escape of carbon dioxide. All analyses were performed in duplicate and if adequate checks were not obtained additional duplicates were run.

RESULTS. *The basis for measurement of amino nitrogen reabsorption.* The creatinine clearance has been used as a measure of glomerular filtration rate under conditions favorable for maximum accuracy, namely, constant plasma concentrations between 30 and 40 mgm. per 100 cc. The amount of amino nitrogen filtered at the glomeruli per unit of time is calculated as the product of the plasma concentration in milligrams per cubic centimeter and the rate of glomerular filtration in cubic centimeters per minute. The amount excreted is equal to the product of urinary concentration in milligrams per cubic centimeter and urine flow in cubic centimeters per minute. The amount reabsorbed is obviously the difference between these two quantities.

The characteristics of the reabsorptive system for glycine amino nitrogen. The essential data from 3 experiments on dog 1 are given in table 1. It may be seen by referring to experiment 12 of this table that normally more than 98 per cent of filtered amino nitrogen is reabsorbed. As the plasma concentration is raised by the infusion of glycine the amount reabsorbed fails to increase in proportion to the amount filtered and excretion becomes appreciable. Both excreted and reabsorbed moieties continue to increase until the latter reaches a limiting value of approximately 21 mgm. per minute. Further increases in the amount filtered are accompanied by proportionate increases in the amount excreted. When the amount filtered exceeds that amount necessary to saturate the reabsorptive

TABLE 1

Experiments on a normal dog which show the relationship between the amount of glycine amino nitrogen filtered and the amounts reabsorbed and excreted

All infusions at a rate of 5 cc. per minute. Dog 1; 18.7 kgm; S.A. 0.72 sq.m.

EXPER. NO.	GLOMERULAR FILTRATION RATE	URINE FLOW	AMINO NITROGEN					CLEARANCE RATIO AMINO-N CREATININE
			Plasma conc.	Urine conc.	Filtered	Excreted	Re- absorbed	
Infusion 0% glycine								
12	cc./min.	cc./min.	mgm.%	mgm. %	mgm./min.	mgm./min.	mgm./min.	
	82.1	9.85	3.76	.050	3.09	0.05	3.04	0.02
	85.2	9.80	3.65	0.67	3.11	0.07	3.04	0.02
Infusion 2% glycine								
	94.2	3.80	8.25	3.16	7.77	0.12	7.65	0.02
	97.2	4.40	9.25	7.90	9.00	0.35	8.65	0.04
Infusion 3% glycine								
	109	5.30	12.2	37.6	13.3	2.00	11.3	0.15
	107	5.20	13.2	46.4	14.1	2.41	11.7	0.17
Infusion 4% glycine								
	110	5.20	17.5	84.3	19.3	4.39	14.9	0.23
	109	5.30	19.0	96.3	20.7	5.10	15.6	0.25
Infusion 6% glycine								
7	123	5.70	27.4	234	33.7	13.4	20.3	0.40
	118	5.45	27.2	235	32.1	12.8	19.3	0.40
	120	4.90	27.2	252	32.7	12.4	20.3	0.38
Infusion 3% glycine								
	118	3.50	23.7	245	28.0	8.58	19.4	0.31
	114	3.30	22.4	224	25.6	7.39	18.2	0.29
	115	3.35	20.8	197	24.0	6.60	17.4	0.28
	116	3.05	20.2	188	23.5	5.74	17.8	0.25
	112	2.90	19.7	185	22.1	5.36	16.7	0.24
Infusion 10% glycine								
8	109	12.1	47.7	250	52.0	30.2	21.8	0.58
	100	10.4	49.5	278	49.5	28.9	20.6	0.58
	95.5	9.30	52.3	309	50.0	28.7	21.3	0.58
	99.3	9.40	52.8	324	52.5	30.4	22.1	0.58
Infusion 5% glycine								
	95.8	6.20	46.5	375	44.5	23.3	21.2	0.52
	95.8	5.50	43.5	377	41.6	20.8	20.8	0.50
	97.5	5.35	41.5	365	40.5	19.5	21.0	0.51
	94.2	4.90	40.0	360	37.7	17.7	20.0	0.47

system (between 30 and 35 mgm. per minute filtered) either gradual increase or gradual decrease of plasma concentration is without effect on the quantity reabsorbed (expt. 8).

All of our observations on dogs 1 and 2 are plotted in figures 1 and 2. Less extensive observations on two other dogs are in complete qualitative agreement. These figures illustrate well the gradual approach to a maximal rate of reabsorption for glycine amino nitrogen (T_m) and the consequent gradual increase in excretion. These characteristics serve to distinguish sharply the amino nitrogen reabsorptive mechanism from the glucose system. According to Shannon and Fisher (1938), glucose reabsorption keeps pace with filtration until the maximal

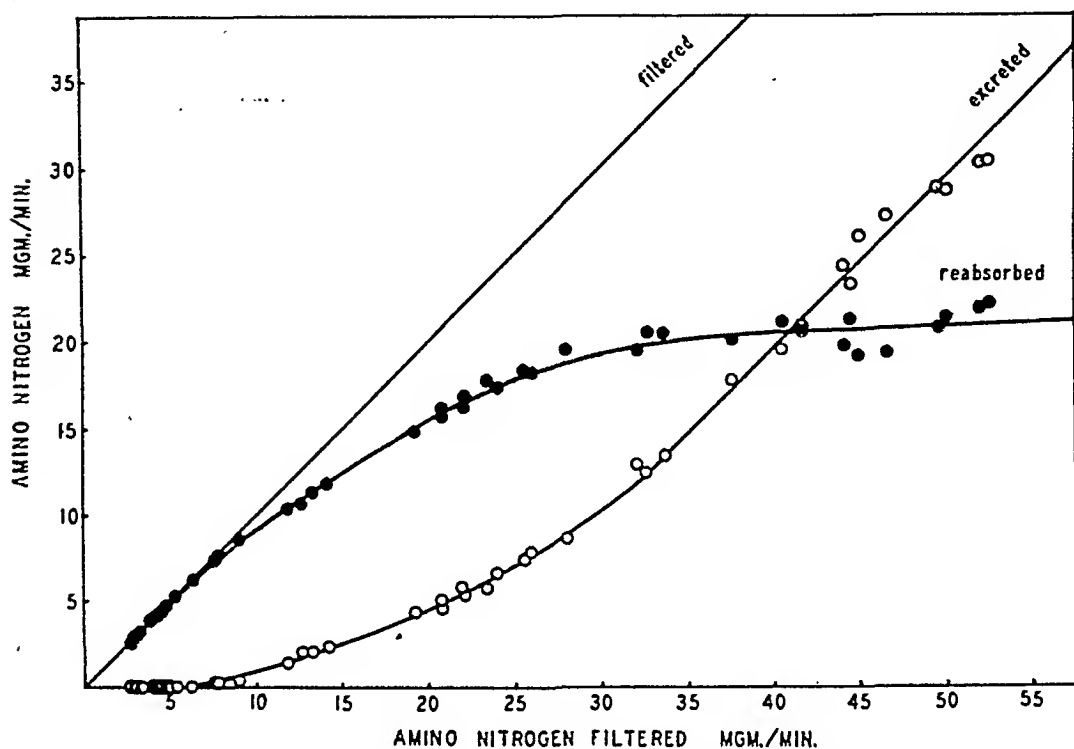


Fig. 1. The renal reabsorption and excretion of glycine amino nitrogen as a function of the quantity filtered in dog 1.

rate of reabsorption is attained. Excretion then begins and increases exactly in proportion to the excess filtered.

The amino nitrogen reabsorptive system appears to be no less stable and reproducible than the glucose system. The experiments shown in figures 1 and 2 were carried out over a period of 3 months and the limited scatter of the data is evidence of stability of the mechanism. The maximal reabsorptive capacity for glycine amino nitrogen, however, is characteristic for a given animal. For four dogs the values are 13, 17, 21 and 23 mgm. per minute. All dogs were between 16 and 19 kgm. in weight with surface areas of about 0.7 sq.m. Thus the maximal reabsorptive capacity in mongrel dogs is not a simple function of surface area or body weight. The reabsorption of amino nitrogen was in no way conditioned in these experiments by the reaction of the urine, for pH measurements showed only

negligible variations within limits of pH 6.3 and pH 7.2, and seemed more correlated with urine volume than with amino acid content.

Competition between glycine and creatine for a common reabsorptive system. Tubular reabsorption of creatine is indicated by its absence from the urine under normal conditions and by the progressive increase in clearance as the plasma creatine concentration is raised (Pitts, 1934). Recalculation of the data presented in that paper shows that at plasma concentrations up to 100 mgm. per 100 cc., the quantity reabsorbed still increases in the face of marked excretion. This superficial resemblance of the reabsorptive processes for creatine and glycine and the fact that, chemically, creatine may be considered as a substituted methyl glycine, suggested the possibility that a single tubular transfer mechanism might

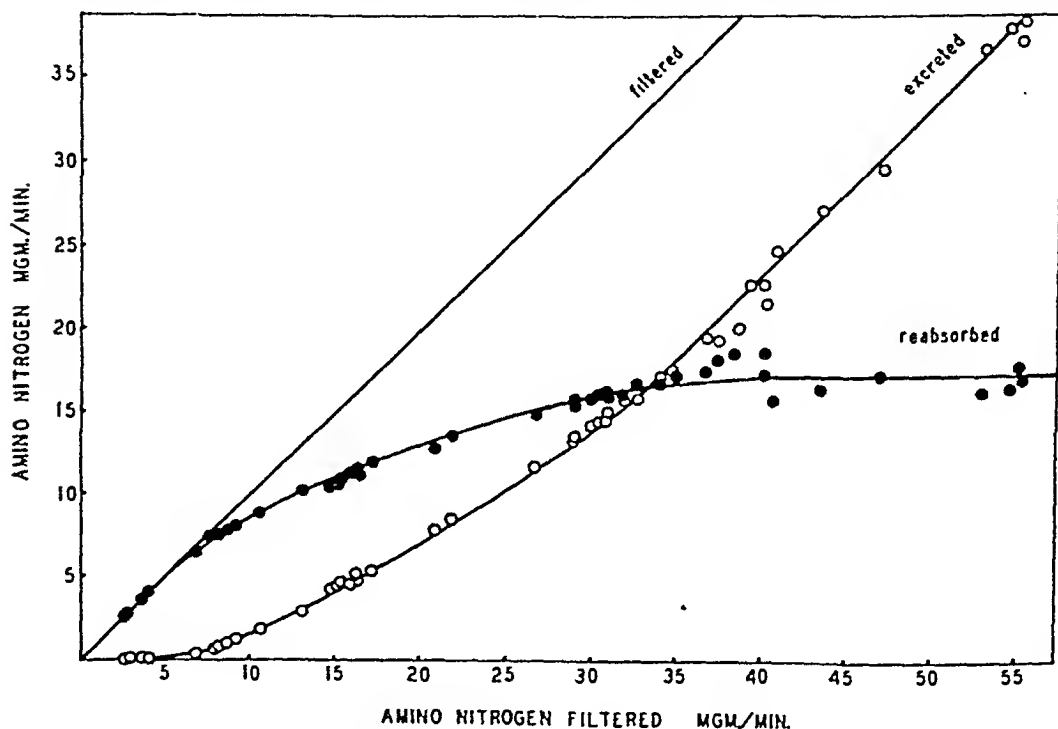


Fig. 2. The renal reabsorption and excretion of glycine amino nitrogen as a function of the quantity filtered in dog 2.

be common to the two substances. The experiments presented in table 2 on dog 3 indicate that the prediction is correct. In experiment 21, the concentration of creatine in the plasma was raised to such a level that accurate analyses could be made in the presence of creatinine. In the first two periods, at normal plasma amino nitrogen levels, 2.7 mgm. of creatine were reabsorbed per minute. As plasma amino nitrogen concentrations were raised by progressively increasing the glycine content of the infusions, the amount of creatine reabsorbed diminished. In the last two periods of experiment 21, upon saturation of the tubular transfer system with amino nitrogen, creatine reabsorption dropped to zero. In experiment 22 the infusion of larger amounts of creatine approximately doubled the quantity reabsorbed. Again reabsorption dropped to zero on administering large

amounts of glycine. These experiments indicate that glycine and exogenous creatine compete for a common reabsorptive system. Experiment 23 duplicates experiment 22 as nearly as possible except that both creatine and creatinine were omitted from the infusions. Creatinine was omitted in order to analyze, with some fair degree of accuracy, the endogenous apparent creatine of plasma. Since the glomerular filtration rate was unknown, amounts filtered and reabsorbed could not be calculated. However, an increase in the amount excreted from 0.008 mgm. per minute in the control periods to 0.251 mgm. per minute after glycine, is indicative of the fact that glycine and endogenous apparent creatine are reabsorbed by the same mechanism. These experiments should well disturb those groups of investigators who study precursors of creatine by feeding various amino acids and note only the elimination of excess creatine in the urine. In fact the use of such a method of approach in any metabolic study cannot be too heartily condemned, for it neglects possible direct alterations of renal function.

The corollary of experiment 23, namely, increasing plasma creatine to high levels and studying normal amino nitrogen excretion has been performed with completely negative results. This is interpreted by us as suggesting a much greater affinity of amino nitrogen than of creatine for some common link of the reabsorptive chain. It is not unreasonable to presume a rather low affinity for creatine in view of the relatively insignificant amounts reabsorbed at even the highest plasma levels.

Alterations in renal function produced by glycine. Claims of acute renal damage from the administration of large amounts of amino acids have been made (Newburgh and Marsh, 1925). Since our animals are all alive, we have had no opportunity to examine the kidneys microscopically. Functionally, however, neither acute damage as evidenced by hematuria, nor chronic damage as evidenced by reduced glomerular filtration rate or tubular reabsorptive capacity have been observed. We have performed some 16 experiments on dog 1, during the course of which a total of over one kilogram of various amino acids has been administered, without evidence of any chronic reduction of renal function. The reduction in reabsorption of creatine shown in table 2 cannot be assigned to non-specific renal damage for it is manifest in periods 3 and 4 of experiment 21 at plasma amino nitrogen concentrations within a range attainable after a meat meal.

However, significant functional variations in glomerular filtration rate and minimum effective renal plasma flow are routinely observed on administration of glycine, as is evident on inspection of table 3. The feeding of meat, casein and glycine or the parenteral administration of the latter substance increases the rate of glomerular filtration in the dog (Pitts, 1935). Hiatt (1942) has shown that meat feeding in the seal causes an even greater increase in glomerular function and a marked increase in renal plasma flow. The p-amino hippuric acid clearance given in table 3 is accepted as a measure of minimum effective renal plasma flow (Finkelstein, Aliminosa and Smith, 1941). It may be seen that elevation of plasma amino nitrogen by infusion of glycine is followed by progressive increase in glomerular filtration rate and renal plasma flow. At moderately elevated plasma

Experiments on a normal dog which illustrate competition between creatine and glycine amino nitrogen for a common reabsorptive mechanism

All infusions at a rate of 5 cc. per minute. Dog 3; 17.5 kgm; S.A. 0.76 sq.m.

EXPER. NO.	GLOMERULAR FILTRATION RATE	AMINO NITROGEN			CREATINE		
		Plasma conc.	Excreted	Reabsorbed	Plasma conc.	Excreted	Reabsorbed
Infusion 0.5% creatine; 0% glycine							
21	cc./min.	mgm. %	mgm./min.	mgm./min.	mgm. %	mgm./min.	mgm./min.
	74.0	5.50	0.04	4.04	31.9	20.9	2.7
	74.8	5.50	0.06	4.02	29.5	19.4	2.7
							2.7
Infusion 0.5% creatine; 2% glycine							
	79.2	9.11	0.60	6.62	26.5	19.2	1.8
	77.6	10.2	0.94	6.97	25.5	18.2	1.6
							1.7
Infusion 0.5% creatine; 4% glycine							
	74.4	15.6	2.99	8.61	22.0	15.7	0.7
	72.0	18.2	3.67	9.45	22.7	15.5	0.9
							0.8
Infusion 0.5% creatine; 6% glycine							
	66.0	31.0	8.30	12.2	23.1	15.5	0.0
	67.6	36.4	10.4	14.2	23.5	15.9	0.0
							0.0
Infusion 2.0% creatine; 0% glycine							
22	59.7	4.17	0.02	2.47	109	59.9	5.2
	64.2	4.03	0.02	2.57	108	63.6	5.9
	64.8	3.66	0.02	2.35	112	66.3	6.3
							5.8
Infusion 2.0% creatine; 7.5% glycine							
	62.6	47.1	17.1	12.4	114	71.4	0.0
	54.5	55.4	18.1	12.1	118	65.2	0.0
	51.7	63.3	19.4	13.4	123	64.1	0.0
							0.0
Infusion 0% creatine; 0% glycine							
23		3.72	0.02		0.84	0.006	
		3.64	0.02		0.82	0.010	
		3.51	0.03		0.80	0.008	
						0.008	
Infusion 0% creatine; 7.5% glycine							
		46.1	17.1		0.98	0.232	
		53.2	19.4		1.02	0.250	
		59.8	22.4		1.06	0.271	
						0.251	

levels, renal plasma flow increased disproportionately to glomerular filtration rate so that filtration fraction decreases (ratio of creatinine to p-amino-hippuric acid clearance). At still higher levels filtration fraction returns to the usual figure of about 0.30.

TABLE 3

Experiments on a normal dog which indicate the types of renal functional change which result from glycine infusion

All infusions at a rate of 5 cc. per minute. Dog 2; 18.7 kgm.; S.A. 0.71 sq.m.

EXPER. NO.	GLOMERULAR FILTRATION RATE	URINE FLOW	PLASMA CONCENTRATION		CLEARANCE		AMINO NITROGEN	CLEARANCE RATIO	
			Amino nitrogen	p-Amino hippurate	Amino nitrogen	p-Amino hippurate	Re- absorbed	Amino-N Creatinine	Creatinine Hippurate
Infusion 0% glycine									
11	cc./min.	cc./min.	mgm. %	mgm. %	cc./min.	cc./min.	mgm./min.		
	63.4	8.10	4.19	3.12	0.72	210	2.63	0.01	0.30
	64.7	8.15	4.18	2.85	0.86	206	2.67	0.01	0.31
Infusion 2% glycine									
	74.5	6.90	9.08	2.20	3.30	275	6.46	0.04	0.27
	78.2	6.30	10.2	2.14	5.83	267	7.23	0.08	0.29
Infusion 4% glycine									
	81.0	8.20	25.6	1.60	31.2	341	12.8	0.38	0.24
	82.0	7.67	26.7	1.53	31.3	361	13.5	0.38	0.23
Infusion 6% glycine									
	82.5	9.40	35.1	1.70	38.0	300	15.5	0.46	0.27
	81.1	9.35	38.2	1.79	39.2	286	16.0	0.48	0.28
Infusion 9% glycine									
13	86.6	20.0	63.1	1.92	60.1	264	16.7	0.69	0.33
	85.1	18.0	65.0	1.92	57.2	262	18.0	0.67	0.33
	82.2	18.9	67.1	1.92	57.0	254	16.8	0.69	0.32
	75.4	16.9	70.0	1.95	52.4	244	16.2	0.70	0.31
Infusion 4.5% glycine									
	72.1	11.2	64.5	2.10	45.6	228	17.1	0.63	0.32
	69.2	10.1	62.7	2.19	43.1	218	16.4	0.62	0.32
	66.6	9.0	60.6	2.22	41.0	215	15.7	0.61	0.31
	69.0	9.9	57.8	2.26	39.2	222	17.2	0.56	0.31

It has been commonly observed that glomerular filtration rate tends to drop when plasma amino nitrogen is maintained elevated for long periods of time. This is evident in experiment 8 of table 1 and experiment 13 of table 3. Note also in experiment 13 that renal plasma flow diminishes. It is rather surprising that

filtration rate and blood flow are not more seriously depressed considering the severe signs of somatic and visceral disturbance. These signs include vomiting, dilatation and fixation of the pupils, weakness and muscular inco-ordination. Recovery is relatively rapid and essentially complete within a few hours of the end of the experiment. To date no fatalities have occurred as a result of amino acid infusion, although, as described in the next section, near fatalities have occurred.

Independence of amino nitrogen and glucose reabsorption. The reabsorptive systems for glycine and glucose are similar in so far as both show a limitation of maximal transfer capacity. They are different in the way that the limit is approached. To decide whether we are dealing with two distinct systems or with differences in the way two substances are handled by a common system, experiments were performed similar to those on creatine. Only to a degree have our experiments been successful. Saturation of the reabsorptive system with glucose has no effect on reabsorption of normal amino nitrogen. Saturation of the reabsorptive system with glycine amino nitrogen has no effect on reabsorption of normal glucose. Phlorizin in amounts sufficient to produce complete glycosuria does not increase the normal amino nitrogen excretion. These experiments are consonant with the view that the reabsorptive mechanisms are different. However, when the plasma concentrations of glucose and glycine were raised simultaneously to saturation levels, a serious collapse of filtration rate occurred. Associated with this collapse was an approximately equivalent reduction in both glucose and amino nitrogen reabsorptive capacities. Toxic manifestations of high plasma glucose and amino nitrogen were extreme, including coma and a rigidity of a decerebrate type. In one instance the animal was put aside for later autopsy, only to recover after a few hours with no residual signs of renal impairment. The depression of reabsorptive capacity in these experiments we attribute to circulatory collapse and complete closure of some glomeruli, with consequent reduction in the number of tubules contributing to the reabsorptive capacity of the kidney, and not to competition for a common reabsorptive system.

DISCUSSION. Shannon and Fisher (1938) have contributed a simple explanation of those cellular processes which impose a limitation on tubular reabsorption of glucose. With certain modifications, these concepts may be applied to reabsorption of glycine amino nitrogen. There is postulated a stable cellular component B , present in fixed amount, with which amino nitrogen A in the tubular fluid enters into combination in the course of reabsorption. The decomposition of this compound AB to deliver A into the peritubular interstitial fluid is a first order process (fig. 3). If sufficient A is present to completely transform all of B into AB , the rate of transfer across the cell becomes constant and limited by the velocity of this reaction and by the amount of AB present. This concept explains equally well a maximal reabsorptive capacity for glycine and glucose. Of course substance B is different in the two systems.

Shannon and Fisher have assumed for glucose that this second reaction proceeds rather slowly in relation to the rate of attainment of equilibrium in the first reaction, namely, the combination of A with B to form AB . If the postulate

is reversed, namely, the second reaction proceeds rapidly in relation to the rate of attainment of equilibrium in the first, the gradual approach to a limiting tubular reabsorptive capacity for amino nitrogen finds ready explanation. Under conditions such that free B exists in the cell (incomplete saturation of the reabsorptive system), the amount transferred is limited by the effective rate of combination of A (glycin) with B to form AB . Assuming total B ($B + AB$) to be constant, rate of transport will depend upon the concentration of A and the specific velocity of its combination with B . The lower this velocity of combination the more gradually will the reabsorptive system be saturated. The higher this velocity

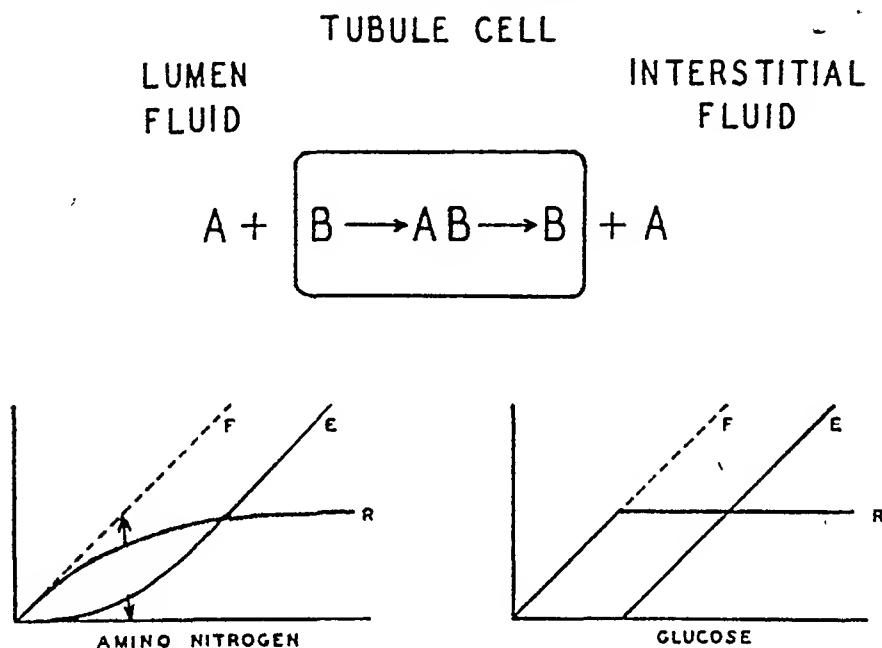


Fig. 3. A schematic representation of the cellular processes governing the reabsorption of amino nitrogen. B is a stable cellular component present in limited and fixed amount with which substance A (amino nitrogen) in the lumen fluid combines during reabsorptive transfer to the peritubular interstitial fluid. The properties of the system are discussed in the text.

the more nearly will the amino nitrogen system behave like the glucose system (note arrows in the inset graph for amino nitrogen in fig. 3).

Shannon (1938) has given an alternative explanation of what may be fundamentally a similar phenomenon, namely, the gradual approach to saturation of the reabsorptive system for the sugar xylose. Xylose and glucose are reabsorbed by a common cellular mechanism, yet the characteristics of the two processes are dissimilar in that xylose is excreted at all plasma concentrations and the amount reabsorbed appears to increase in proportion to concentration over a wide range. Shannon assumes that equilibrium conditions are maintained for xylose no less than for glucose. Differences in the two reabsorptive processes are then explicable in terms of differences in equilibrium constants for the combination of the two sugars with the common cellular element B . An equilibrium

constant for xylose, some 1500 times that for glucose would approximately account for the different characteristics of their reabsorptive processes. One might assume an intermediate equilibrium constant to account for a maximal reabsorptive capacity which is reached more gradually for glycine than for glucose and more rapidly for glycine than for xylose. We are however inclined toward the first explanation presented as a result of our further studies on the reabsorption of other amino acids to be presented subsequently.

The competition between creatine and glycine for a common reabsorptive mechanism depends upon the substance *B* common to both reabsorptive systems. If combined with glycine it is unavailable to creatine, and as a consequence creatine reabsorption is proportionately reduced. However, the affinity of glycine for this common link must be greater than that of creatine, for large amounts of creatine do not depress amino nitrogen reabsorption.

SUMMARY

1. The tubular reabsorption of amino nitrogen has been assessed at various arterial plasma levels obtained by the infusion of glycine.

2. Amino nitrogen is reabsorbed by an active mechanism which exhibits a limitation of transfer capacity. The maximal rate of reabsorption is attained rather gradually and as a consequence no sharp renal threshold exists.

3. Creatine is reabsorbed by the same system. Competition between creatine and amino nitrogen for a common link in the reabsorptive chain brings about a reduction in the amount of creatine reabsorbed at elevated plasma amino nitrogen levels.

4. As a result of this competition for a common renal reabsorptive mechanism, studies of creatine precursors based on the feeding of amino acids and the measurement of excess creatine eliminated in the urine are rendered suspect.

5. While the administration of large amounts of glycine produces marked immediate alterations in renal function, we have seen no evidence of any chronic damage.

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RAPID ACCLIMATIZATION TO WORK IN HOT CLIMATES

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The problem of man's acclimatization to heat has claimed the attention of numerous physiologists. There seems to be general agreement about some of the changes that take place, although the extent of these changes and the reasons for them are less certain. Almost all observers agree that working men in hot climates have higher blood volumes and interstitial fluid volumes than the same men in cold climates. Forbes, Dill and Hall (1940) found this change to be slight; Bazett et al. (1940) found it to be great and to come on early. Other changes associated with acclimatization to heat have been reported: Scott et al. (1940) noticed a temporary increase in resting cardiac output as well as an increase in peripheral circulation; Lee (1940) found a decline in heart rate, and Burton (1940) observed an initial increase in the heat exchange followed by a return to the control level. Many observations have been made upon the changes in the sweat in acclimatization to heat. Dill and his collaborators (1933, 1937, 1938) showed a decrease of 50 per cent in the concentration of sodium chloride in the sweat of working men, which decrease Johnson et al. (1943) have attributed to the lower body temperatures resulting from acclimatization. Associated with these changes are the obvious alterations in the total amount of sweat, the increase in water ingested and the decrease in the urine excretion, as noted by Adolph and Dill (1938). Most observers agree that the process of acclimatization results in an increased output of sweat under a given set of conditions—Haldane (1935), Moss (1923), Adolph and Dill (1938), Dill (1938) and Winslow et al. (1938)—in addition to the obvious increased sweating when the change is made from one climate to another. On the other hand, Knipping (1923) reported that sweating decreases with acclimatization—a discrepancy undoubtedly due to differences in the activities of the subjects.

In addition to these specific and easily measurable changes, certain others of a more general character are known to occur. The comfort zone of temperature is higher in summer than in winter (Yaglou, 1927) and with acclimatization a man's ability to do prolonged physical work in the heat improves markedly (Robinson et al., 1941). The present study represents an attempt to measure quantitatively this improvement in the ability to do work and particularly to follow its time relations. How quickly does the improvement occur and how soon is it lost when returning to a cooler environment? Some effort was made also to determine to what physiological variables this improvement is related.

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PROCEDURE. The experiments were carried out in Boston during February and March in an artificially heated room where the temperature was about 104°F. and the humidity 23 per cent. In no single experiment reported here on any given man did the average room temperature vary by more than 0.7°F. nor the humidity by more than 2 per cent from the mean for that man's entire series of experiments. The work consisted of walking on a motor driven treadmill at 3.5 m.p.h. on a grade of 5.6 per cent for four of the subjects and 4.0 per cent for the other man. With two exceptions all walks for each man were at the same speed and grade and for the same length of time, the duration being determined by the length of time required to exhaust him at the beginning. These walks will be referred to below as the standard hot room walks. The program was continued until the men had trained for 10 to 23 days. The exceptions to the standard walk were by subjects SR and WH who on one occasion after acclimatization continued walking at the standard grade and speed for three times the duration of their original walks. On another occasion these same two subjects walked on a higher grade (9 per cent) for their standard length of time. In addition to the work periods, subjects HB, ET, and SR spent an additional 3 to 4 hours each day in the hot room making observations on other subjects.

The heat produced by the body in the walk on a 5.6 per cent grade is about 30 per cent greater than the heat produced by men in the regular army march on the level with pack. The men used as subjects were five laboratory workers, all of whom were accustomed to walking on the treadmill under ordinary laboratory conditions but none of whom had been exposed to heat since the preceding summer. All of them were in such good condition that they completed a 40 mile hike on the road in one day. During all experiments included in this paper they wore standard army summer cotton trousers, shirt, tie, woolen socks and service shoes. On about half of their days of acclimatization, subjects HB, ET, and SR wore clothing which varied from this costume and the resulting data are not included in this paper. Pulse rate was determined by palpation, rectal temperature by clinical thermometer, and skin temperature by four thermocouples fixed on the skin, one each on chest, back, thigh and upper arm. The rate of water loss from skin and lungs was determined by weighing the nude subject before and after work. Metabolic weight loss was subtracted from the weight differences. Oxygen intake was determined once during each experiment by collecting and measuring the subjects' expired air and analyzing samples in the Haldane apparatus.

RESULTS. The effects of acclimatization on the heart rates, rectal temperatures, and skin temperatures of the men during the work experiments in the hot room are shown in figure 1. Except for ET, who walked on the lower grade, the men approached heat exhaustion in the early experiments, with high skin temperatures, rectal temperatures of 103° to 104°F., and heart rates averaging 178 beats per minute during the last 20 minutes of work. It is obvious from the data in figure 1 that repetitions of the constant task increased the comfort and ease with which the work could be done. It is significant that about 80 per cent of the improvement noted is found to have occurred in the first 7 days of exposure. After 23 days of acclimatization by walking in the hot room two men (SR and

HB), in order to determine the completeness and effectiveness of their acclimatization, repeated the standard walk with the room temperature lowered to 72°F. In the walks in the cooler room the men reached heat balance with their rectal

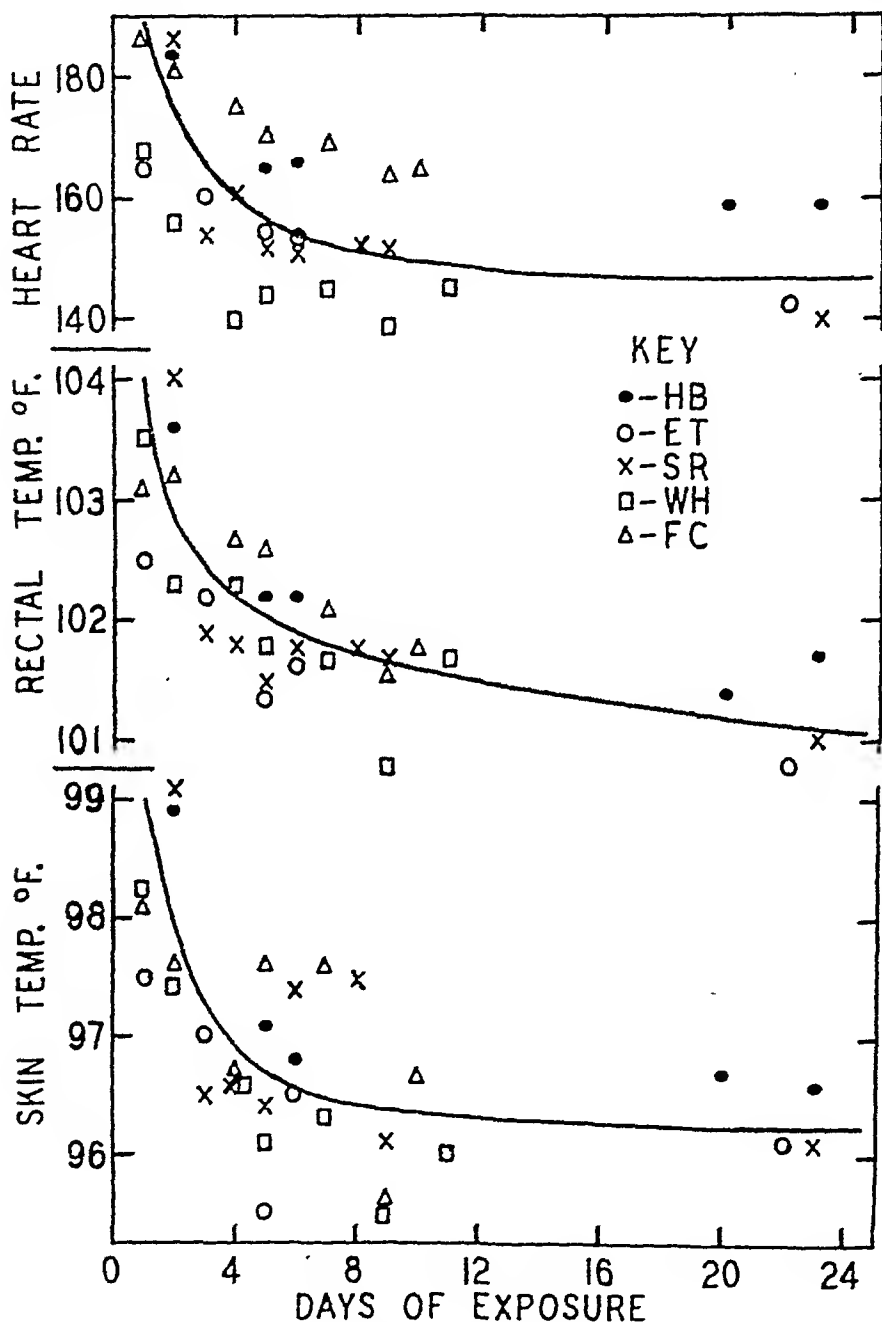


Fig. 1. Acclimatization to heat as shown by the lowering of body temperatures and heart rates of men in the standard work experiment (room temperature 104°F., humidity 23 per cent). On some of the days of exposure the men wore clothing which varied from the standard costume and the resulting data are omitted from this figure.

temperatures and metabolic rates the same as they were in their hot room experiments at the end of acclimatization (table 1). In the cool room experiments, radiation and convection played a large part in the dissipation of body heat, and,

therefore, loss of weight by evaporation from skin and lungs was small (table 1). In the hot room the men even absorbed heat from the room. Under these conditions, evaporation became great enough to dissipate all the heat produced within the body plus that absorbed by it (table 1). The fact that skin temperatures, rates of sweating, and heart rates were higher while the acclimatized men were working in the hot room experiments than when they were in the cool room shows that the mechanisms for heat dissipation were under greater stress in the

TABLE 1

Comparison of the performance of the standard work by men in the cool room and in the hot room after they were acclimatized to heat

SUBJECT	ROOM CONDITIONS		METABOLISM	BODY TEMP.		SWEAT	HEART RATE
	Temp.	Rel. Hum.		Rectal	Skin		
	°F.	per cent	Cal./hr.	°F.	°F.	kgm./hr.	
SR	104.1	23	453	101.0	96.1	1.41	140
	72.1	32	450	100.8	87.5	0.58	128
HB	103.8	25	482	101.4	96.6	1.43	159
	71.9	32	485	101.3	87.8	0.61	134

TABLE 2

The effects of acclimatization on the metabolic rate and the rate of sweating of men in the standard work experiments

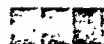
(Room temperature 104°F., relative humidity 23 per cent, rate of walking 3.5 m.p.h. on a 5.6 per cent grade)

SUBJECT	BODY SURFACE	BODY WEIGHT		METABOLISM		SWEAT	
		Before acclimatization	After acclimatization	Before acclimatization	After acclimatization	Before acclimatization	After acclimatization
	m ²	kgm.	kgm.	cal./hr.	cal./hr.	kgm./hr.	kgm./hr.
HB.....	1.88	69.1	68.5	526	483	1.47	1.43
ET*.....	2.00	72.6	72.5	500	482	1.47	1.53
SR.....	1.75	65.4	64.3	540	453	1.40	1.41
WH.....	1.79	66.9	66.4	574	506	1.44	1.45
FC.....	2.00	84.9	86.2	686	686	1.35	1.57
Average....	1.88	71.8	71.6	565	522	1.43	1.48

* Subject ET walked on a 4 per cent grade as compared with a grade of 5.6 per cent for the other subjects.

hot room. (Nielsen (1938) has also observed the constancy of the rectal temperature of a man working at a constant rate in widely different environmental temperatures and has shown the adjustments to be due to evaporative and circulatory changes.)

During acclimatization subject FC experienced an increase in his rate of sweating in the standard work experiments but showed no significant change in his metabolic requirement for the work (table 2). The other four subjects were more



successful in becoming acclimatized than FC—they underwent decreases in metabolism yet showed no significant changes in their rates of sweating in the standard work experiments (table 2). The effect of acclimatization on the maximal capacity for sweating was determined by increasing the intensity of work in the hot room in one experiment on each of subjects SR and WH. Table 3 shows

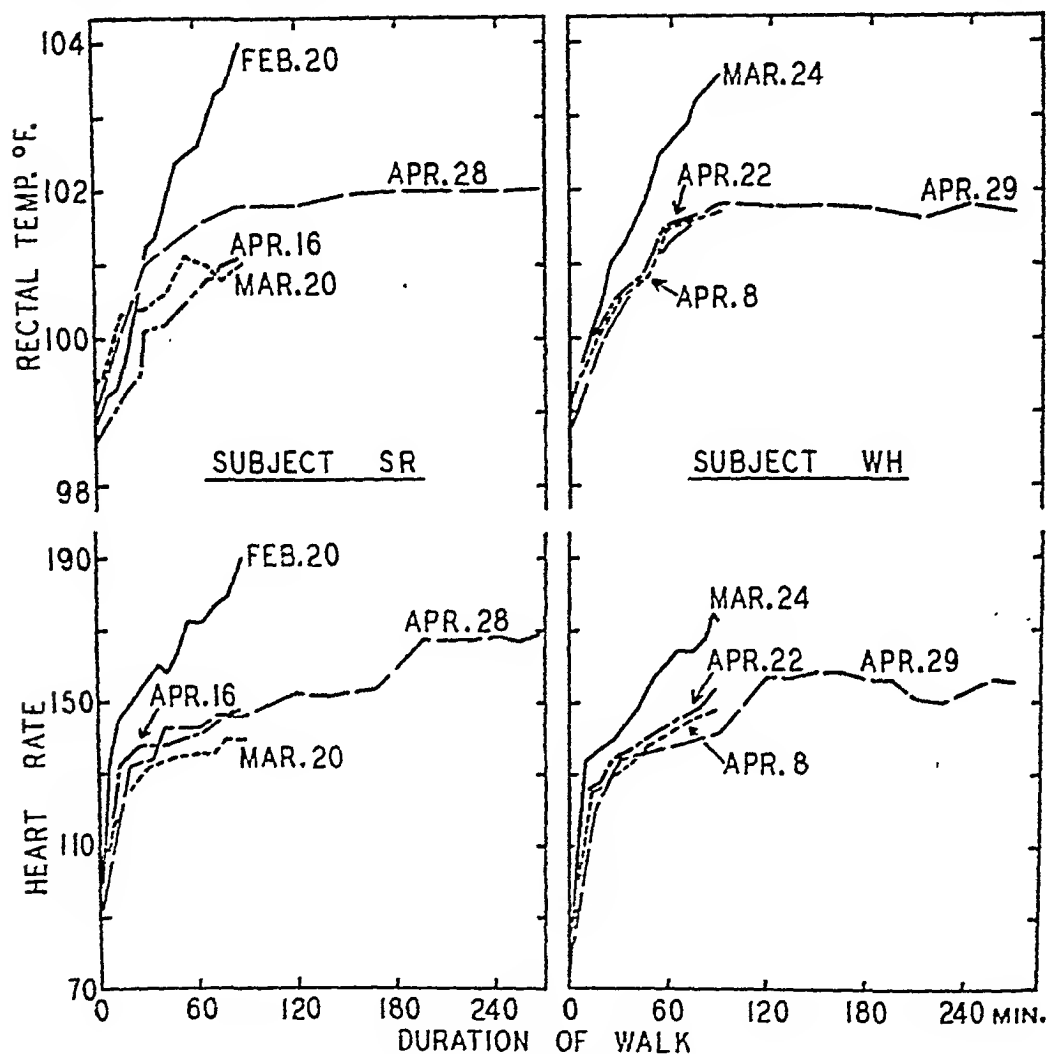


Fig. 2. Acclimatization to heat as shown by the lowering of body temperatures and heart rates of two men walking at 3.5 m.p.h. on a 5.6 per cent grade (room temperature 104°F., humidity 23 per cent).

a. Subject SR was acclimatized by 23 exposures between February 20 and March 20. After March 20 his only exposures were on April 16 and 28.

b. Subject WH was acclimatized by 11 exposures between March 24 and April 8. After April 8 his only exposures were on April 22 and 29.

that there was an average increase of 21 per cent in the actual capacities of these two men to sweat.

It is of practical importance to know how much more work men can do in the heat after acclimatization than they could before. Accordingly, after subjects WH and SR were acclimatized, tests were conducted in which they walked at the standard rate and grade in the hot room with ease for 4½ hours. This was three

times as long as in their original exhausting walks. It should be noted that no rest periods were taken in these long experiments. Figure 2 gives a comparison of the performances before and after acclimatization by these two men. Their rectal temperatures in the long walks were only moderately elevated and remained constant throughout the last 3 hours. Their heart rates never reached critical levels. On the other hand, in the original experiments they were completely exhausted in $1\frac{1}{2}$ hours with extremely high body temperatures and heart rates.

TABLE 3

The increase in the maximal rate of sweating with acclimatization

Before acclimatization maximal sweating was induced by the standard walk and after acclimatization by raising the intensity of the walk to 3.5 m.p.h. on a 9 per cent grade. (Room temperature 104°F., relative humidity 23 per cent.)

SUBJECT	BEFORE ACCLIMATIZATION		AFTER ACCLIMATIZATION	
	Sweat	Rectal temp.	Sweat	Rectal temp.
	kgm./hr.	°F.	kgm./hr.	°F.
SR.....	1.40	104.0	1.85	103.0
WH.....	1.42	103.5	1.66	102.0
Average.....	1.41	103.8	1.76	102.5

TABLE 4

Loss of acclimatization to standard work in the hot room

The skin temperatures and heart rates represent the average of several determinations made during the last 20 minutes of each work experiment

SUBJ.	BEFORE ACCLIMATIZATION			AFTER ACCLIMATIZATION				LOSS OF ACCLIMATIZATION			
	Rectal temp.	Skin temp.	Heart rate	Period of acclim.	Rectal temp.	Skin temp.	Heart rate	After expos. ceased	Rectal temp.	Skin temp.	Heart rate
	°F.	°F.		days	°F.	°F.		days	°F.	°F.	
FC.....	103.1	98.1	186	10	101.8	96.7	164	15	102.9	97.6	176
HB.....	103.6	99.0	184	23	101.4	96.6	159	24	102.9	97.1	178
SR.....	104.0	99.1	186	23	101.0	96.1	140	26	101.2	96.1	146
ET.....	102.5	97.5	168	21	100.9	96.1	143	28	101.2	94.5	150
WH.....	103.5	98.2	168	11	101.7	96.0	144	13	101.7	96.3	148
Ave.....	103.3	98.4	178	18	101.4	96.3	150	21	102.0	96.3	160

It is also important to know how long the state of acclimatization to heat lasts after exposures are discontinued. Three of the five subjects in this study were only slightly less efficient in the standard hot room experiments 2 to 3 weeks after stopping the exposures than at the end of acclimatization. This can be seen from the heart rates and body temperatures of the men (table 4). It is shown graphically for subjects WH and SR in figure 2. It should be noted that these two men performed their $4\frac{1}{2}$ hours' walks in the hot room 3 and 5 weeks respectively after the period of acclimatization was over. Subjects FC and HB, however, in repeating their walks 2 and 3 weeks respectively after stopping the exposures to heat had lost much of their acclimatization (table 4). This is probably related to the

fact that these two men had greater difficulty acclimatizing in the first place and never reached as complete a state of acclimatization as the other three subjects.

DISCUSSION. The most striking results of these experiments were: 1, the rapidity with which acclimatization occurred (about a week); 2, the shortness (1 to 1½ hrs.) of the daily periods of work in the heat needed to produce acclimatization; 3, the retention of acclimatization by some of the subjects for periods of 3 weeks or more after exposures to heat ceased, and 4, the completeness of the acclimatization as evidenced by the fact that after acclimatization the men could maintain heat equilibrium in the standard grade of work about as well in the heated room as in the same walk in a cool room.

These observations are obviously of great practical importance to industry and are even more important where rapid changes of climate by active men are necessary. Men who are already in good physical condition can be expected to work effectively within a few days after they start work in a hot climate; by a few relatively short daily exposures to work in artificially heated rooms they can be prepared for working immediately after they arrive in a hot climate. This process of acclimatization is not a substitute for the prolonged period of training necessary to produce good physical condition, but it is essential before men already in good physical condition can work effectively in the heat.

Additional experiments are needed to determine whether even more rapid acclimatization might be achieved by shorter, harder, more frequent work periods in the heat or by more prolonged daily periods of work.

The mechanisms involved in the improvement in performance of these men as they became acclimatized to the heat are not entirely clear from our data. The marked changes in heart rate and skin temperature of the men during acclimatization indicate that circulatory adjustments played an important part in the adaptation. From evidence cited above, these circulatory changes probably involved changes in peripheral circulation as well as in blood volume and tissue fluid volume. Since the men's skin temperatures during work in the heat were higher before than after acclimatization it is obvious that heat exchange by radiation was more favorable to the men before than after acclimatization. In the four men who showed decreases in their metabolic requirements for the standard work the average difference between the heat production during work before and after acclimatization was a decrease of 54 Calories per hour. This approximately equaled the average decrease of 51 Calories per hour in the rate of accumulation of stored body heat during work which occurred as a result of acclimatization. Since there was not a training effect the decrease in energy requirement for the work in the heat must be considered as a result of acclimatization as well as a contributor to it. The high rates of metabolism of these men during work in the early exposures to heat were not due to lack of training but apparently were associated with the high environmental temperature and the men's high body temperatures. This is evidenced by the fact that at the time of the first exposure to the heat all of the men were in good physical condition and were experienced in walking on the treadmill. HB had less experience on the treadmill than the others and yet he showed no training effect in his metabolic

requirement for the standard work—479 Cal./hr. in the cool room before exposure to heat, compared with 485 Cal./hr. after acclimatization to heat. These values are practically the same as similar determinations on him in the heat after acclimatization and significantly lower than in his initial exposure to heat (table 2).

One factor which contributed to the acclimatization of FC was his increased rate of sweating in the standard work experiments. Only 40 per cent of his increase in sweat secretion during acclimatization had to evaporate to account for his decrease in accumulation of body heat during work. It should be pointed out that in these experiments a considerable and unknown fraction of the sweat dripped off of the men and some accumulated in the clothing. The other subjects continued throughout acclimatization to sweat at about the same rate as in the initial experiments. The reason that these men did not increase their rates of sweating in the standard walks as did FC is that they showed greater decreases in body temperature during the walks as acclimatization proceeded (table 4). In their cases the work no longer elicited maximal rates of sweating (table 3).

The difference between FC and the other subjects in the manner and completeness of acclimatization to the heat may have been due in part to the fact that, although he was in good physical condition, he is a large, stocky man. He had 43 kgm. of body weight per square meter of body surface, as compared with 36 to 37 kgm. for each of the other four subjects. Since heat production in walking is proportional to body weight and heat dissipation depends largely on surface area, it is obvious that FC was handicapped in his heat regulation as compared with the other men (Robinson, 1942). In relation to surface area, FC sweated almost as much as the other subjects, whereas in relation to body weight, he sweated distinctly less than the others even after the gain he made during acclimatization. It is interesting that, although the rapid improvement in temperature regulation and comfort during the first few days of acclimatization may be accompanied by either a decrease in heat production or an increase in sweating or both, the continued slower improvement took place in these subjects without a further change in either of these processes. Therefore, the improvement in temperature regulation cannot be entirely dependent upon them.

SUMMARY

During the winter, experiments were carried out in which men walked on a motor driven treadmill from 1 to 1½ hours a day in a room where desert conditions were simulated. When the men first began to take the walks the work was severe enough and sufficiently long to bring on symptoms of heat exhaustion.

The comfort and ease with which the men repeated the same walks which originally exhausted them increased rapidly during about 7 days and thereafter more slowly up to 23 days.

The heart rates of the men during the latter part of the walks declined from an average of 178 in the beginning to 155 on the seventh day.

The average skin temperature and rectal temperature of the men at the end of the work experiments declined from 98.4 to 96.5 F. and from 103.4 to 101.7 F. respectively during the same period.

This rapid improvement in temperature regulation during the first 7 days amounted to about 80 per cent of the entire improvement in 23 days. It was accompanied by an increase in the rate of sweating in one man and decreases in metabolic rate during work in the others. The slow improvement in temperature regulation occurring after the seventh day was not accompanied by continued lowering of metabolic rate nor by increase of sweating during the experiments. However, the capacity for sweating in harder work than the standard experiments did increase.

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CONSECUTIVE CHANGES IN CUTANEOUS BLOOD FLOW, TEMPERATURE, METABOLISM AND HEMATOCRIT READINGS DURING PROLONGED ANESTHESIA WITH MORPHINE AND BARBITAL¹

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In the course of studies under prolonged anesthesia of the peripheral circulatory reactions during hemorrhage and shock, decreased cutaneous blood flow, rise in rectal temperature and oxygen consumption and fluctuations of hematocrit readings were observed during the first six hours after induction of the anesthesia which complicated the interpretation of the changes in peripheral circulation and metabolism occurring in the development of the shock state. These observations necessitated a study of the serial changes following the anesthesia alone, the results of which are presented in this paper.

METHODS. The effects of the various anesthetics were studied in 47 dogs weighing 7 to 20 kgm. The dogs were either unoperated or had inserted tracheal, arterial and venous cannulas. The following anesthetic combinations were given: 1, morphine sulphate and chlorallosane; 2, morphine and sodium pentobarbital; 3, morphine and sodium barbital; 4, sodium pentobarbital alone; 5, sodium barbital alone, or 6, morphine alone. In the following pages, where morphine and chlorallosane or a barbiturate are referred to, the morphine will be called the primary and the chlorallosane or barbiturate the secondary anesthesia. In all experiments the primary anesthetic dose of morphine was 2.0 to 4.4 mgm/kgm. The solutions administered were the standard preparations used in this laboratory for anesthesia. The morphine was given subcutaneously in the form of a 2 per cent solution in sterile distilled water. The sodium barbital was given as a 10 per cent solution prepared from "barbital sodium Merck, U.S.P.," the sodium pentobarbital was given as a 5 per cent solution prepared from "sodium pentobarbital powder, U.S.P.XI," purchased from Premo Pharmaceutical Laboratories, Inc.; and the chlorallosane was given as a 0.5 per cent solution. The solutions were freshly prepared and were dissolved either in sterile distilled water or in pyrogen-free water and administered either intravenously or intraperitoneally. Except for a slower action in the latter no significant difference was noted in either the dose required or the effects produced by the alternate routes of administration.

Subcutaneous temperatures were recorded in degrees centigrade with iron-constantin needle thermocouples inserted subcutaneously, and the body temperature from an iron-constantin couple inserted 10-15 cm. into the rectum, by

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means of a Leeds and Northrup 6 point Micromax recorder. The couples were automatically connected in turn to the recorder. The temperature of any one couple was recorded once every six minutes. The instrument is stated to have an accuracy of $\pm 0.3^{\circ}\text{C}$, but by keeping it carefully adjusted we were able to maintain an accuracy of the order of $\pm 0.2^{\circ}\text{C}$. Subcutaneous temperatures were found to be about 1° higher than surface temperatures but parallel to them, and less subject to error due to the fluctuating contact of the couple with the skin. The hair was clipped over the region of insertion of the thermocouples. In most of the experiments the animals were unheated and the rectal temperature was allowed to vary at will. In a few experiments, however, the rectal temperature was kept constant by varying the heating current supplied to two lamps placed under the animal board. Dark blinds were used at the windows and all drafts were avoided in order to maintain as even an environmental temperature as possible. The temperature of the air in the vicinity of the dogs was recorded with one of the needle thermocouples. Hematocrit readings were made of arterial blood with Wintrobe tubes. The results are expressed as cell volume per cent of total volume. One *point* is used as meaning 1 cell volume per cent. Oxygen consumption was measured with a recorder of the Sanborn blower type connected to a tracheal cannula. Blood pressure and heart rate were recorded by suitable optical manometers (1) or with a mercury manometer.

RESULTS. I. *Rectal temperatures in unanesthetized dogs.* Rectal temperatures, measured in 21 dogs immediately upon bringing them into the experimental laboratory from the animal house, ranged from 37.5° to 39.7° , with an average of 38.5° . The temperature ranged slightly higher during the summer than in the winter. These temperatures correspond well with those recently reported by Federov and Shur (2) and by Friedman and Bennett (3). Of 6 trained, unanesthetized dogs, 4 maintained their initial rectal temperature for the 1 to 4 hrs. during which they were kept lying quietly, but in 2 the rectal temperature dropped approximately 0.5° during the first 0.5 hr. before becoming stable at 38.8° and 39.0° . As shown in the first part of figure 2 this decline in rectal temperature was associated with a prominent rise in the subcutaneous temperature of the paws.

II. *Subcutaneous and rectal temperatures. a. Morphine plus chloralose, sodium barbital or sodium pentobarbital.* Two dogs were given morphine sulphate followed in 3 hrs. and in 1 hr. by 50 and 73 mgm/kgm. respectively of chloralose; 6 were given morphine followed in 0.25 to 1.25 hrs. by 160 to 200 mgm/kgm. of sodium barbital and 7 received morphine followed in 0.75 to 2.5 hrs. by 25 to 30 mgm/kgm. of sodium pentobarbital. The results were essentially the same with all three anesthetic combinations. Those from a typical experiment are illustrated in figure 1. In this experiment the rectal temperature had fallen to 35°C by the time the temperature recorder was connected. It reached a minimum of 32° five hours after giving the secondary anesthetic, after which it rose and stabilized at 38.6°C . At this time the animal was still deeply anesthetized. When recording began the temperatures of the skin over the thorax,

over the knee and on the dorsum of the hind paw were within 2° to 3° of the rectal temperature. Throughout the experiment the former two maintained about the same relation to the rectal temperature but about 2.7 hrs. after giving the barbitol the temperature of the skin on the dorsum of the paw began to decline at the rate of 1.5° per hour. Prominent shivering such as was noted in this experiment was seen during the rise of the rectal temperature in 5 of these experiments.

In 10 of this group of 15 dogs, receiving morphine plus chloralose or a barbiturate, lowering of the subcutaneous temperature of the paws by 4.7° to 11.5° was noted within 0.75 to 6 hrs. after giving the secondary anesthetic. The rectal temperatures ranged from 34° to 38.8° when the drop in subcutaneous temperature occurred. In all but 1 of these dogs the rectal temperatures started to rise about the same time as, or shortly after, the subcutaneous temperature dropped. Within 3 to 11 hrs. after giving the secondary anesthetic, the rectal temperature of all 15 of the dogs had risen to 38.6° – 40.5° . In 4 of these dogs the rectal temperature continued to rise for 10 to 40 hrs. reaching levels of 39.5° to 42.6° . In 3 of the animals the subcutaneous temperature of the paws rose abruptly after the rectal temperature reached 39° to 40° .

The somewhat diphasic character of the above responses suggests that the morphine and barbiturate may have partially antagonistic reactions. The diphasic nature is more accentuated in those experiments in which recording was begun prior to the administration of morphine and in which the interval separating the primary and secondary anesthetic injections was increased. The results in a typical experiment of this type are reproduced in figure 2. Within six minutes after giving morphine the rectal and thoracic subcutaneous temperatures began to decline and the subcutaneous temperatures of the paws began to rise. The latter reached a maximum at 0.3 hr. and then dropped at a rate of 2° to 3° per hour. Again within 6 minutes after the intravenous injection of chloralose the subcutaneous temperatures of the paws suddenly rose, associated with a further fall of rectal temperature and of the subcutaneous temperatures of the ear and thorax. When the rectal temperature reached 36° the subcutaneous temperature of the paws began to decline at a rate of 5° to 6° per hour, and within 1.3 hrs. the rectal temperature began to rise. No shivering was seen in this experiment. The subcutaneous temperature of the fore-paw reached a minimum of 26° within 2.5 hrs. and the rectal temperature rose to a maximum of 38.9° within about 5 hrs. after administration of the secondary anesthetic. The subcutaneous temperature over the thorax remained within 1.5° to 2.5° of the rectal temperature. The subcutaneous temperature of the ear declined during the rise in rectal temperature. This was twice replaced by intervals of elevated temperature when small intravenous injections of sodium barbitol were given.

II-b. *Sodium barbitol and sodium pentobarbitol alone.* Four dogs were anesthetized with 200 mgm./kgm. of sodium barbitol, and two dogs with 25 and 35 mgm./kgm. of sodium pentobarbitol alone. In all 6 of the animals anesthetized with a barbiturate the initial drop in rectal temperature was small

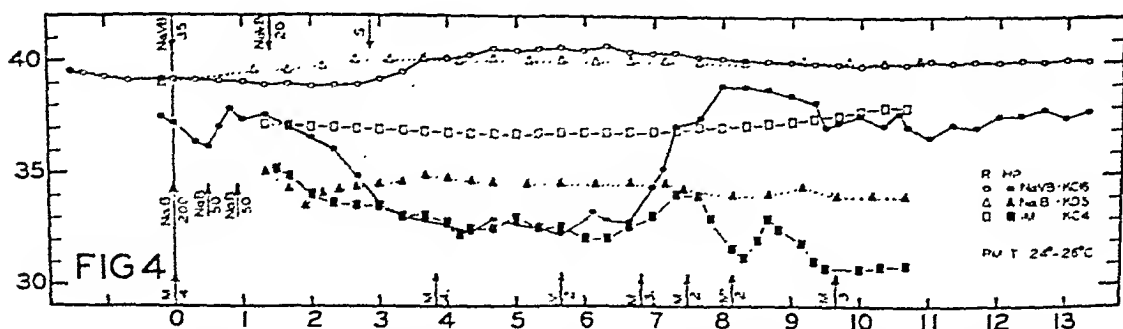
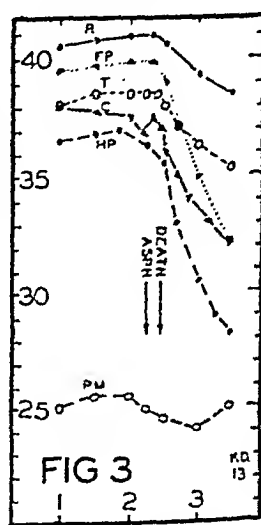
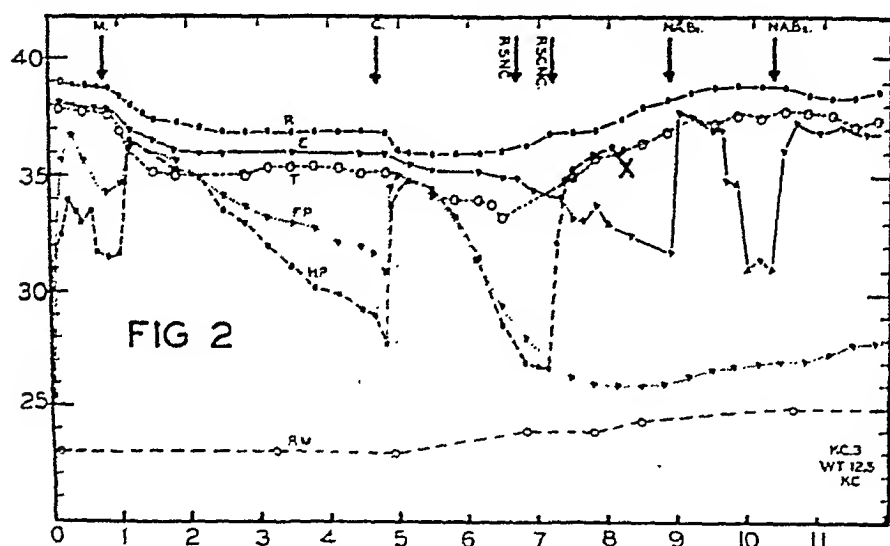
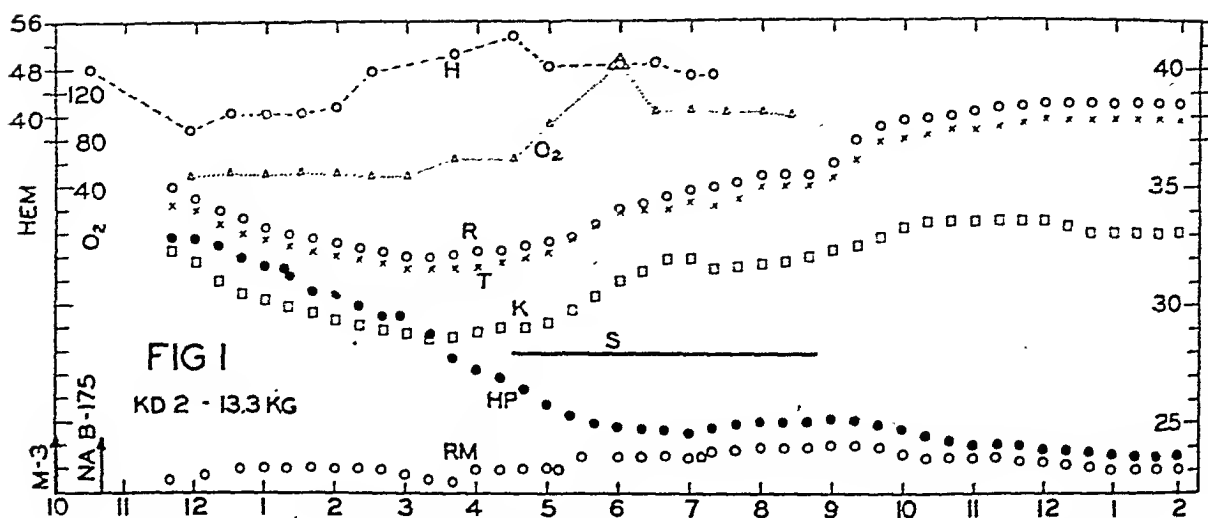


Fig. 1. Consecutive changes in rectal and subcutaneous temperatures, hematocrit readings and oxygen consumption in a dog anesthetized with morphine and sodium barbital. *H*—hematocrit reading, scale at left (*HEM*) = cell volume per cent of total volume; *O*₂—oxygen consumption, scale at left = *O*₂ consumption in ml/min; *R*—rectal temperature, *T*—subcutaneous temperature over thorax, *K*—subcutaneous temperature in region of knee, *HP*—subcutaneous temperature on dorsum of hind foot, *RM*—air temperature near animal, scale for temperature at right in degrees centigrade; *S*—duration of observed shivering; *M-3*—subcutaneous injection of 3 mgm/kgm. of morphine; *NA-B-175*—intravenous injection of 175 mgm/kgm. of sodium barbital; abscissal scale—time in hours. Note all subsequent charts are drawn to the same scale as this chart.

(0.5° to 1.5°C) and in from 6 to 12 hrs. the rectal temperature had risen to 39.0 to 40.5°C and to 40.0° to 41.7°C after 15 to 28 hrs. Despite the smaller initial drop in rectal temperature there was in many of the barbiturate experiments a decline in the subcutaneous temperature preceding the rise of rectal temperature. The degree of lowering of the rectal temperature was usually less prominent and subsequent rise more marked than when morphine had been given preceding the barbiturate. Either alone or with morphine, sodium pentobarbital caused an earlier and more prominent rise of rectal temperature than sodium barbital. The results in two typical experiments are reproduced in figure 4.

II-c. *Morphine alone.* All but 1 of the 12 dogs, in which morphine alone was used, were anesthetized sufficiently to allow surgical procedures. This required from 4 to 20 mgm. per kgm. initially and a maintenance dose of 0.7 to 4.2 mgm. per kgm. per hour. In 8 of the 10 dogs studied at room temperatures of 23° to 26° the rectal temperature fell to between 34° and 37° within 1.5 to 4 hrs. Four showed no subsequent rise or decline and 4 showed a rise of less than 0.5°. In these dogs the subcutaneous temperature of the hind paws declined 1.2° to 10.2° within 1 hr. and 20 min. to 5 hrs. This drop in subcutaneous temperature occurred usually when the rectal temperature was around 35.9° to 37.8°, although in two instances it occurred at higher rectal temperatures. One animal was heated and in one the rectal temperature rose spontaneously. In

Fig. 2. Rectal and subcutaneous temperatures in a dog anesthetized with morphine and chloralose, with prolonged interval between the two injections. Effect of cutting vasomotor nerves on subcutaneous temperature. *E*—subcutaneous temperature of outer surface of ear; *FP*—subcutaneous temperature of dorsum of fore-paw; *HP*—subcutaneous temperature of dorsum of right hind-paw; *X*—cessation of registration of subcutaneous temperature of hind paw; *M*—subcutaneous injection of 3.2 mgm/kgm. of morphine; *C*—intravenous injection of 50 mgm/kgm. of chloralose; *RSNC*—section of right saphenous nerve; *RSCNC*—section of right sciatic nerve; *NAB₁* and *NAB₂*—intravenous injections of 80 mgm/kgm. of sodium barbital. Temperature scale at left in degrees centigrade; other lettering and scales as in figure 1.

Fig. 3. Rate of decline of subcutaneous temperature upon cessation of circulation. *C*—subcutaneous temperature of skin over calf; *ASPH*—asphyxiation; *DEATH*—cessation of cardiac and respiratory action as result of the asphyxiation. Other lettering and scales as in figures 1 and 2.

Fig. 4. Superimposed curves of rectal temperatures and subcutaneous temperatures of hind paw in dogs anesthetized with sodium pentobarbital, with sodium barbital and with morphine alone. Open symbols—rectal temperatures; solid symbols—subcutaneous temperatures of dorsum of hind paw. Circles—temperatures from dog anesthetized with sodium pentobarbital, intravenous injections of 35 and 20 mgm/kgm. indicated by upper row of arrows, *S*—onset of shivering in this animal, weight—18 kgm. Note in the original this record extended for another 7 hrs. with a rise of rectal temperature to 41.5°C 3 hrs. after the end of the reproduced segment of the record followed by a gradual decline to normal. The dog was returned to the animal cage at the end of this time and remained in good health. Triangles—temperatures from dog anesthetized with sodium barbital, intravenous injections of 200, 50 and 50 mgm/kgm. at times indicated by middle row of arrows, weight 18.5 kgm. Squares—temperatures from dog anesthetized with morphine, injections of 4, 4, 2, 3, 2, 2, and 3 mgm/kgm. at times indicated by bottom row of arrows, weight 10 kgm. Room temperature for all three experiments ranged between 24 and 26°C. Scales as in figures 1 and 2.

both animals when the rectal temperature reached 39° the subcutaneous temperature of the paws suddenly rose several degrees. In the 2 dogs studied at room temperature of 28° to 30° the rectal temperature remained within 0.5° of the pre-anesthetic level and the subcutaneous temperature remained within 3° of rectal temperature throughout the experiment. Shivering was not noted in any of the experiments with morphine alone. The results in one experiment are reproduced in figure 4.

III. *Effects of environmental temperature.* Room temperatures ranged in the different experiments from 21.5° to 30° , but most of the experiments were performed at room temperatures of 24° to 26°C . The variation in any one experiment was rarely more than 1.5° to 2.0° . Changes of subcutaneous and rectal temperature were observed in some animals at all environmental temperatures. However, with room temperatures of 28° to 29°C or higher it was observed that there was rarely much decline in subcutaneous temperature with or preceding the rise of rectal temperature; that the rectal temperature of dogs anesthetized with morphine did not decline appreciably; and that the rectal temperature of dogs anesthetized with a barbiturate rose higher than it did with dogs at lower room temperatures.

IV. *Evaluation of subcutaneous temperature changes in terms of blood flow.* Sudden maximal increase in blood flow was induced by cutting the sciatic nerve in 3 animals anesthetized with morphine alone and in 3 animals anesthetized with morphine and chloralose during periods of lowered and of moderately elevated subcutaneous temperature. Prior cutting of the saphenous nerve had no effect, but in each instance after section of the sciatic nerve the subcutaneous temperature of the hind paw began to rise within six minutes and within 20 to 30 min. was within 1° to 2° of the rectal temperature. An example of this reaction is seen in figure 2.

The effect of sudden cessation of blood flow was studied by recording temperatures for 0.5 to 2 hrs. after death in 15 animals. When at death there was a difference of 10° to 15° between the recorded temperature and the room temperature the following initial rates of decline were observed: rectal temperature 1.0° to 2.0° per hour, thoracic subcutaneous temperature 2.0° to 3.0° per hour, subcutaneous temperature over the mid-region of the extremities— 2° to 4° per hour and over the paws 4° to 8° per hour. These data are illustrated in figure 3. When the thorax was opened, allowing the thoracic contents to drop away from the chest wall, the rate of decline of the thoracic subcutaneous temperature approached that of the paws.

Discussion. When the temperatures of the room and of the subcutaneous tissues are remaining constant an equilibrium exists between the rate of elimination of heat from the skin and the rate at which heat is being brought to the skin by the circulation and by direct conduction from the deeper structures. If in a given region heat is supplied almost solely by the blood and if the rate of circulation is low the equilibrium temperature is at or only slightly above room temperature, whereas if the rate of circulation is rapid the subcutaneous temperature will approach the internal body temperature as reflected by the rectal temperature.

Temperatures in between these two extremes will then, as suggested by Burton (4), indicate intermediate rates of flow. This arrangement appears to apply, however, only to areas of the skin, such as the ear and paws, which lie over regions of low heat content or ability to generate heat, and which are dependent almost entirely upon heat conducted to them by the blood stream. As indicated above, apparently the skin in the proximal portions of the extremities over large muscle masses, and especially the skin over the thorax and abdomen, receive heat by direct conduction from the deeper structures at such a rapid rate that the equilibrium temperature is determined by this mode of conduction rather than by the influence of any changes in the rate of heat conduction by the blood stream. As a result, as shown in figures 1 and 2, despite evidence of marked reduction in blood flow in the skin over the paws and ear which presumably would be accompanied by similar changes in the remainder of the skin, the subcutaneous temperature of the latter regions of the skin remains quite close to the rectal temperature. A corollary of this observation is the conclusion that, while marked generalized cutaneous vasoconstriction would reduce the heat loss from the skin over the extremities and similar regions, it would have only a minimal effect on the heat loss from the skin over the trunk and large muscle masses.

The interpretation of changes of blood flow by means of the recorder used in these experiments is dependent upon having a sufficient difference of temperature between the room temperature and the rectal temperature that small changes in blood flow can be detected readily. As a practical rule we have found that a minimum of about 15° is desirable for this purpose. The onset of a change of blood flow is probably accompanied by beginning change of subcutaneous temperature with a lag of only 30 sec. to a minute (4). However, because of the construction of our recorder the apparent latency in our records may be anywhere up to six minutes. Interpretation of the degree of change of blood flow after the assumption of a new rate of flow can be made only in a roughly qualitative manner until the subcutaneous temperature has again come to equilibrium. During the period of changing subcutaneous temperature an estimate of the new rate of blood flow can be made only by comparing the *rate of change* of subcutaneous temperature with the rate of change which normally occurs after maximal increase of flow such as is induced by section of the vasomotor nerve (the sciatic for the hind paw) or maximal reduction of flow as by sudden death. On the basis of such comparisons we conclude that in many instances maximal increase of flow is induced by the administration of either morphine or barbitol and that when a reduction of blood flow accompanies a rise of rectal temperature it is likewise very close to maximal.

The initial increase in cutaneous blood flow and the resulting increased heat loss plus a reduction of the spontaneous muscular activity was probably responsible for the initial drop in rectal temperature. Their occurrence may have been due to a depression of that center in the hypothalamic region which prevents fall of body temperature (Ranson, Fisher and Ingram (5)). The barbiturates and morphine seemed to potentiate each other slightly in causing

this initial effect. The subsequent decrease of cutaneous blood flow, the shivering and the rise of body temperature to and frequently well above normal with the barbiturates may have been due to a differential wearing off of the anesthetic depression of that part of the temperature-regulating center which prevents a fall of body temperature, while the depression of the center which prevents an overshooting of the body temperature (5) was still present. On the other hand, it could be equally well argued that the febrile like reaction, which was similar to that described by Du Bois (6) during the chill stage in man, was due to the presence of pyrogenic substances present in the various powdered preparations used. However, studies were carried out on 6 additional dogs using Abbott's Veterinary Nembutal (sodium pentobarbital) solution which is specially prepared for intravenous use, and the results were identical with those described above. Regardless of whether the sequence of temperature changes is due to the effect of the barbiturate per se or to the presence of an unrecognized pyrogen contaminant, allowance must be made for these changes when studying the peripheral circulation in animals anesthetized with these preparations.

V. *Oxygen consumption.* Throughout periods of anesthesia with morphine alone the oxygen consumption showed minor fluctuations but tended to remain relatively constant or to parallel the rectal temperature. However, as seen in figure 1, with morphine plus one of the barbiturates and with barbital alone there was usually a rapid rise in oxygen consumption to 125 to 200 per cent of the initial value during the period of vasoconstriction and rising rectal temperature, even in the absence of visible shivering. The O_2 consumption often dropped slightly when the rectal temperature became stabilized but remained elevated above the level present immediately after the animal was anesthetized.

Discussion. Using the Meeh-Rubner equation (6) for computing the surface area, $S = \frac{11.2 \times W^{2/3}}{100}$, S = surface area in meters,² W = weight in kgm., and assuming 1 liter of oxygen has a calorific value of 4.8, the metabolic rates under morphine anesthesia ranged from 33.2 to 51.8 Cal/M²/hour; under morphine and sodium barbital the metabolic rates ranged from 23.7 to 34.6; and under sodium barbital alone they ranged from 38.9 to 62.3. These were taken either during the initial depression of rectal temperature or after the rectal temperature had stabilized at a subsequent higher level. They show a rather wide scatter. When, however, the metabolic rates were plotted against the rectal temperatures the spread at any given temperature was much smaller, ranging at 38.5°C between 35 and 51 Cal/M²/hour. Comparison of pairs of readings from the same animal indicated that on the average the metabolic rate increased about 10 per cent per degree centigrade increase of rectal temperature. The discrepancy in the case of those that did not change characteristically with rectal temperature was probably due to increases in muscle tone which were not recognized as shivering. Metabolic rates for trained animals reported in the literature range from 24.6 to 39.2, using the same formula for calculating surface area (7-11). It appears that under morphine alone or morphine and barbital the metabolic rates are within the upper range of those found in unanesthetized animals, while those with sodium barbital alone are higher.

The initial increase in cutaneous blood flow and slight drop in rectal temperature during a barbiturate anesthesia are in agreement with the observations described by Richter and Oughterson (12) by Deuel, Chambers and Milhorat (13) and by Hemingway (14). The subsequent elevation of the rectal temperature to and often above normal has apparently not been previously observed in the dog but was seen in a patient by Edmonson (15). Diminished oxygen consumption has been observed after anesthetization with barbital by Anderson, Chen and Leake (16), with sodium amytal by Dameshek, Myerson and Loman (17) and Shapiro (18), and after delvinal sodium by Peoples and Carmichael (18). However, none of these investigators followed the metabolism for longer than 0.25 to 3 hrs. Increased oxygen consumption has been reported in cats under chloralose anesthesia by Griffiths, Emery and Lockwood (20). The complete sequence of changes such as we have described has not, to our knowledge, been reported.

VI. *Hematocrit readings.* The initial pre-anesthetic hematocrit reading in 19 dogs ranged from 41.1 to 56.4 with an average of 49.6. Within 0.5 hr. after the injection of barbital or pentobarbital the hematocrit reading usually decreased 5 to 11 points below the initial reading taken prior to the injection. This drop coincided with the increase in cutaneous blood flow. The absence of a diminution in three experiments may have been due to the fact that the second sample was not taken soon enough after giving the secondary anesthetic. The hematocrit reading began to increase again within 0.5 to 1.5 hrs. that is, when the rectal temperature was beginning to rise and the subcutaneous temperature to drop. The reading usually became stabilized at 2 points below to 14 points above the initial reading within 2.5 to 5 hrs.; that is, at about the time the subcutaneous temperature reached a minimum; the average in 12 experiments was at 3 points above the initial reading. The serial changes in hematocrit values in several typical experiments are reproduced in figure 5. With morphine alone the hematocrit reading usually showed little change throughout the period of anesthesia. The results in four experiments are reproduced in figure 6. Since, during the stable period the hematocrit readings tended to fluctuate by as much as ± 2 points, it would appear that a change in hematocrit reading induced by an experimental procedure would have to be greater than 2 points in order to be significant.

Discussion. The initial hemodilution confirms the observations of Jarcho (21), but this author apparently did not follow his animals long enough to observe the subsequent hemoconcentration. The explanation of the hematocrit changes cannot be stated with certainty on the basis of our experimental results. However, the association of the hemodilution with the increased blood flow and the hemoconcentration with the reduction of blood flow in the skin suggest that they probably are due to changes in the volume of blood in reservoirs such as the skin and spleen which may hold proportionally more red cells than plasma in the dilated state, and proportionally more plasma in the constricted state (22, 23). The association of such reactions with differential changes in splenic inflow and outflow described by Grindley, Herrick and Mann (24) and in spleen volume found by Seeley, Essex and Mann (25), and the reduction or abolition of the

hemodilution after splenectomy reported by Searles (26) and Adolph and Gerbasi (27) suggest that the spleen may be the chief reservoir in dogs. This is also suggested by the fact that marked hemodilution occurred in animals in which decrease in cutaneous blood flow was prevented by keeping the rectal temperature above normal (see below), and also by the small effects of increase and decrease of cutaneous blood flow upon the hematocrit in animals anesthetized with morphine.

VII. *Effects of artificially maintaining the rectal temperature constant.* In 8 dogs, anesthetized with sodium barbital alone, an attempt was made to avoid

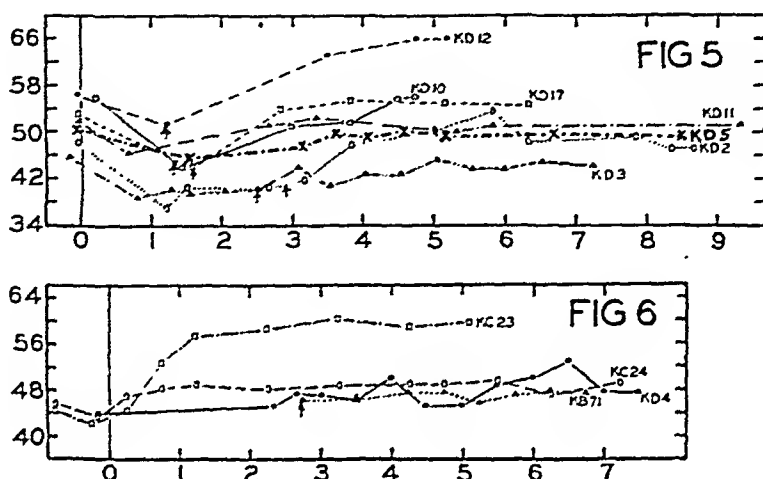


Fig. 5. Superimposed tracings of hematocrit readings from several experiments on dogs anesthetized with sodium barbital. *KD-2*—same experiment as reproduced in figure 1, morphine and sodium barbital, animal not heated; *KD-3*—morphine and sodium barbital, animal warmed sufficiently to prevent a drop in rectal temperature; *KD-5*—same experiment as in figure 4, sodium barbital only, not heated; *KD-10*, *11*, *12* and *17*—dogs anesthetized with sodium barbital alone and warmed sufficiently to maintain rectal temperature at 39°C. Small vertical arrows indicate moment when subcutaneous temperature of hind paws began to drop. This occurred despite the warming in experiments *KD-12*, *KD-10*, *KD-17*, *KD-2*, *KD-3*. Points to the left of the solid vertical line are readings taken prior to administration of anesthetic. Ordinate scale—hematocrit reading in cell volume per cent of total volume. Abscissal scale—time in hours. This figure reproduced to same scale as figures 1 to 6.

Fig. 6. Superimposed tracings of hematocrit readings in four dogs anesthetized with repeated injections of morphine. For details see figure 5.

the fluctuations in temperature, metabolism and hematocrit by preventing the drop in rectal temperature during the first 4 to 6 hrs. by warming the animal board. When the animal was heated only enough to prevent a drop in rectal temperature below that observed at the time of anesthetization the reduction in cutaneous blood flow and augmentation of metabolism still occurred in 2–5 hrs. and the rectal temperature rose above normal. By maintaining the rectal temperature between 39° and 40°C, the fluctuations of rectal and cutaneous temperature and metabolism were minimized, but the characteristic decline and subsequent rise in hematocrit reading still persisted.

VIII. *Blood pressure and heart rate.* The mean arterial blood pressures and heart rates were not studied in all experiments. In those in which they were measured the initial mean blood pressures were highest with sodium barbital alone and with morphine and sodium barbital ranging in 20 experiments from 110 to 190 mm. Hg with an average of 148, and with chloralosane, 140 and 160 mm. Hg. They were lowest with morphine alone (9 expts.) and with morphine and sodium pentobarbital (6 expts.) ranging from 96 to 170 mm. Hg with an average of 130 and 123 mm. Hg respectively. The lowest heart rates were seen with morphine alone and with morphine and chloralosane ranging in 9 experiments from 50 to 90 beats per minutes, with an average of 64 beats per minute. The highest heart rates were found with sodium barbital alone, ranging in five experiments from 180 to 250, with an average of 214; and with morphine plus sodium barbital ranging in six experiments from 116 to 235 with an average of 174. In three experiments with morphine and sodium pentobarbital the heart rates were 100, 141 and 203 beats per minute. The mean blood pressure was practically unaffected by changes in blood flow in the skin, by shivering, or by a rise or decline of rectal temperature. The heart rate remained constant during the interval of declining cutaneous temperature, but not infrequently rose during and following the onset of shivering and rise of rectal temperature in animals anesthetized with one of the barbiturates.

Survival. In view of the interest at the present time in the survival of anesthetized animals after various experimental shock procedures, the following data are presented on the dogs used in this study. No significant difference was noted between the various anesthetic combinations. Twenty-nine of the 47 dogs were used for other experimental studies immediately after the conclusion of the period of temperature study. In so far as it is possible to judge, they remained in good condition up until they were sacrificed at the conclusion of the experimental period by bleeding or asphyxia. The total period of observation of these dogs was 8 to 26 hrs. (average—16 hrs.). Sixteen of the remaining 18 dogs were returned to the animal house in good condition and survived indefinitely; one died after 13 hrs.' anesthesia, apparently because of obstruction to the respiratory passage, and one was sacrificed at the end of a week because of the development of a respiratory infection. The average duration of the anesthesia in these 18 animals was: morphine and barbital or barbital alone—40 hrs.; morphine and sodium pentobarbital or sodium pentobarbital alone—11 hrs.; morphine alone $7\frac{1}{2}$ hrs.

SUMMARY

Roughly qualitative estimations of cutaneous blood flow are readily made in the skin of the paws and ears by recording the subcutaneous temperature with needle thermocouples connected with a Leeds and Northrup micromax recorder. If the subcutaneous temperature is remaining constant the blood flow may be estimated from the relationship of the subcutaneous temperature to the air and rectal temperatures. Subcutaneous temperatures approximating the former indicate minimal blood flow; those near the latter suggest maximal blood

flow. Immediately following a sudden change of blood flow the new rate of flow must be estimated by comparison of the ensuing rate of change of subcutaneous temperature with that observed to occur following the onset of a known change of blood flow. Estimation of the blood flow through the skin over the trunk or over large muscle masses in terms of the subcutaneous temperature is less satisfactory apparently because of the high rate of conduction of heat directly from the deeper structures.

The pre-anesthetic rectal temperature in our dogs ranged from 37.5 to 39.7 with an average of 38.5°C. Anesthesia with morphine alone caused an immediate and frequently maximal increase in cutaneous blood flow and a decline in rectal temperature to 34° to 37°C. These changes were often followed in 1 to 3 hrs. by a sharp reduction in cutaneous blood flow. The latter apparently was secondary to the temperature regulating reactions induced by the drop in rectal temperature. Sodium barbital, sodium pentobarbital and chloralose caused a similar initial increase in cutaneous blood flow and a drop of 0.5° to 1.5° in rectal temperature and a subsequent decrease of cutaneous blood flow; and in addition, induced an increase of oxygen consumption, often associated with shivering, and a rise of rectal temperature to 38.6° to 40.5° within 3 to 11 hrs. Anesthesia with morphine plus barbital accentuated the initial drop of rectal temperature.

Hematocrit readings showed little change with morphine alone, but with either morphine and a barbiturate or one of the barbiturates alone the hematocrit reading dropped 5 to 10 cell volumes per cent within a few minutes after anesthesia and then slowly returned to and often above normal during the period of reduction of cutaneous blood flow and rise of rectal temperature.

Changes in rectal temperature and cutaneous blood flow were minimized by maintaining the rectal temperature between 38° and 39° and were often abolished by elevating the rectal temperature to 39°-40° by warming the animal board. The heating had, however, no significant influence upon the initial decline and subsequent rise of the hematocrit reading in the dogs anesthetized with barbital. Both heart rate and mean arterial blood pressure were higher under sodium barbital anesthesia than under the other anesthetic combinations.

Because of the above sequence of events during the first hours after induction of anesthesia with any of these drugs we recommend that a control period of at least 3 to 4 or more hours elapse following anesthetization before proceeding with experimental studies, if hematocrit, oxygen consumption, rectal temperature, or cutaneous blood flow are to be studied. Such a control period is desirable even when the rectal temperature is being maintained constant by warming the animal.

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METABOLISM OF THE PERFUSED DOG'S BRAIN¹

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Values for the "in vivo" oxygen consumption of the brain found in the literature are usually regarded as rough approximations. These figures are based on indirect measurements of blood flow through the brain made by recording either the internal carotid blood flow with a stromuhr or by measuring the venous return from the brain. The arterio-venous oxygen differences were then used to calculate brain oxygen consumption. The existence of anastomotic branches (see fig. 1) between the internal and external carotid arteries indicates that internal carotid artery blood flow is not identical to cerebral blood flow in the dog; moreover, the external jugular veins of the dog carry blood from other structures of the head as well as the brain.

The gross discrepancy between the "in vivo" and "in vitro" values for brain oxygen consumption has been discussed in a recent review (1). Oxygen consumption figures reported for brain tissue slices or brei provide much useful information; but these figures can hardly be expected to approach the actual values for the intact living brain because of the damage to the tissue from the mincing or slicing, the anoxia incurred during the preparation and the artificiality of the medium in which the tissue is placed.

The present report is concerned with some aspects of brain metabolism obtained with a method of perfusing dog's brains in which the circulation was isolated from other tissues as far as could be ascertained by the injection of dyes.

METHODS. Blood for the perfusion was defibrinated as withdrawn from a large dog which was sacrificed. The lungs were removed to use for oxygenating the blood during the experiment, and the pulmonary artery was cannulated close to its origin. The return flow from the pulmonary veins was collected by tying a large cannula into the left auricular appendage and occluding the bicuspid valve by means of a ligature around the auricular ventricular groove. Saline solution was passed through the lung system to wash out the blood and the lungs were suspended in a large container that served as a moisture chamber. The pulmonary artery was connected to one unit of a double pump of the Dale-Schuster type (2). Blood at 37°C. was pumped through the lung system and passed into a reservoir under the moisture chamber. The blood in the reservoir was covered by a layer of mineral oil to reduce the rate of carbon dioxide loss. From the reservoir, the blood returned to the perfusion pump. The defibrinated

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blood was circulated through the lungs for thirty minutes or more before beginning the head perfusion to remove vasoconstrictor substances (3).

A second dog was prepared for brain perfusion under ether anesthesia. The superior sagittal sinus was exposed by trephining the skull over the vessel and elongating the opening with rongeurs. The carotid arteries were then exposed and all muscles branches of the external carotid ligated, as indicated in figure 1. In order to expose and ligate the internal maxillary arteries it was necessary to approach these vessels through the mouth. Incisions were made just behind and slightly medial to the last molars. Separation of the muscles exposed the maxillary arteries, which were ligated central to their ophthalmic branches. In confirmation of Bouckaert and Heymans (4), we found that when the vertebral arteries are ligated the blood flow through the internal carotid arteries is

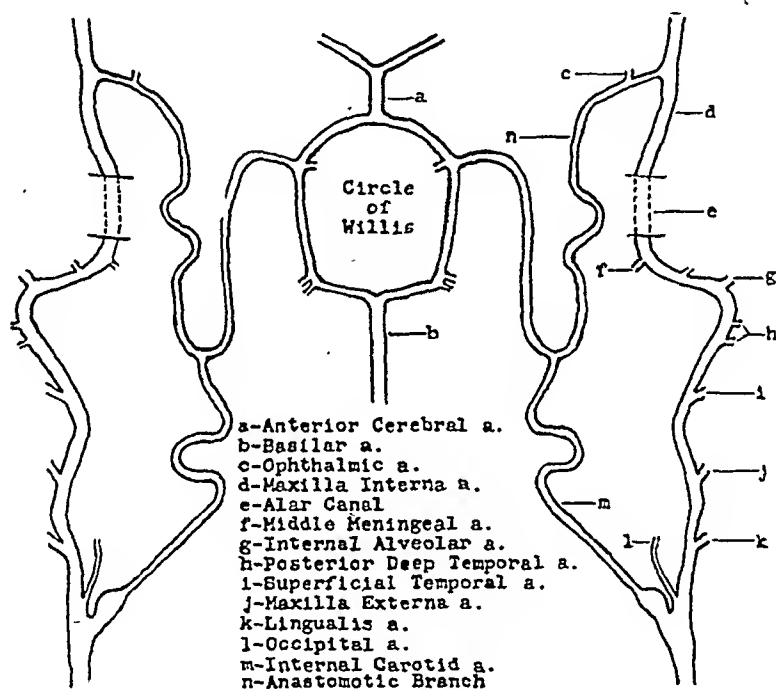


Fig. 1. Diagram of the blood supply of the brain in dogs. Drawn from preparations injected with colored latex.

not adequate to maintain the brain; the larger flow to the brain by way of the external carotid arteries and anastomotic branches (internal ophthalmic arteries) must be functional.

The head was connected to the perfusion pump by cannulating the external jugular veins and the common carotid arteries. This was done on one side at a time, so the circulation to the brain was at no time interrupted. As the head was being connected to the pump, the venous outflow was collected and defibrinated, since part of it came from the perfused dog and had not previously had the fibrin removed. The perfusion pressure was maintained at about the same level as the dog's carotid pressure. A heavy clamp was then tightened about the neck to cut off the blood flow to and from the brain through the vertebral arteries and plexus of veins on the spinal cord surface. Complete occlu-

sion of these arteries and veins proved to be difficult, but was finally accomplished by constructing a suitable clamp. Essentially, this device is a loop of steel cable that can be tightened to compress the vertebral column.

In the course of these experiments it was found that the carbon dioxide concentration in the perfusing blood was below physiological levels due to the necessary exposure of the blood to atmospheric air at two points in its passage through the circuit. Since the concentration of this gas in the blood plays a rôle in the maintenance of normal patency of vessels (5, 6, 7, 8), it was necessary to keep the gas within the normal range. The carbon dioxide tension of the perfused blood was controlled by using the apparatus shown in figure 2.

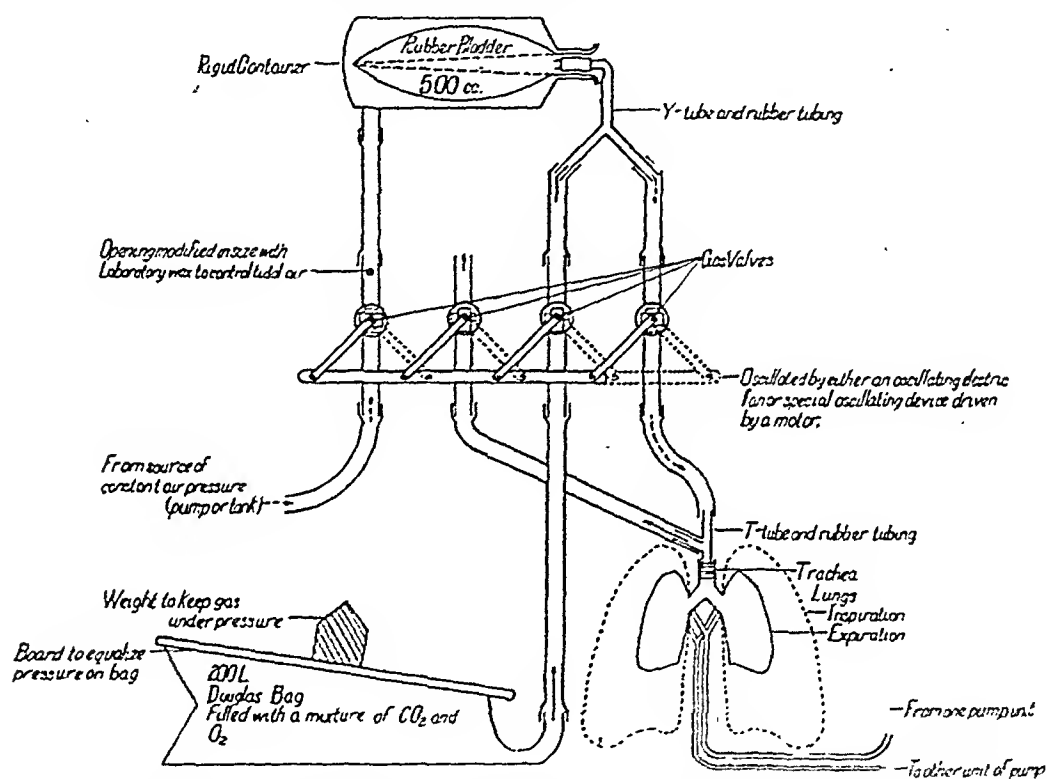


Fig. 2. Apparatus for ventilating the lungs. The solid lines represent the position of the mechanical parts and lungs when air is moving in the direction of the solid arrows. When the oscillating device moves the valves, as represented by the broken lines, the lungs are inflated by air moving as indicated by the broken line arrows.

The perfusion was allowed to proceed for twenty or thirty minutes to eliminate the ether. Blood samples were then simultaneously drawn from the superior sagittal sinus and carotid artery. At the same time the minute blood flow was measured by collecting the venous return from the head in a graduated cylinder. The blood to be used for gas analysis was immediately chilled in ice water. Blood used in the lactic acid and dextrose determinations was treated at once as the respective methods demand. Carbon dioxide and oxygen determinations were made according to the method of Van Slyke and Neill (9), blood sugar according to the method of Hagedorn and Jensen (10), and lactic acid by the method of Miller and Muntz (11) as modified by Barker and Summerson (12).

RESULTS. The results of these experiments, recorded in tables 1 and 2, show

TABLE 1

DOG NO.	TIME	SINUS AND ARTERY	CO ₂	A-S DIFFERENCE	O ₂	A-S DIFFERENCE	DEXTROSE	A-S DIFFERENCE	LACTIC ACID
			<i>vol. per cent</i>		<i>vol. per cent</i>		<i>mgm. per cent</i>		<i>mgm. per cent</i>
1	2:45	S	51.69	4.62	13.13	4.63	128	5	6.2
		A	47.07		17.76		133		6.8
	3:15	S	51.34	3.81	14.11	4.13	154	6	6.5
		A	47.53		18.24		160		6.1
	3:45	S	50.75	3.72	14.41	3.72	155	5	7.4
		A	47.03		18.13		160		6.9
2	2:30	S	30.81	5.29	6.07	5.76	87	11	7.7
		A	25.52		11.83		98		8.1
	3:00	S	34.53	4.91	9.88	5.37	107	7	9.0
		A	29.62		15.25		114		8.7
	3:30	S	32.59	5.43	10.56	5.55	83	8	8.2
		A	27.16		16.11		91		8.5
3	12:25	S	46.82	2.79	14.51	2.96	136	4	7.3
		A	44.03		17.47		140		6.8
	1:00	S	53.41	3.24	9.72	3.68	130	5	7.8
		A	50.17		13.40		135		7.3
4	1:30	S	35.68	5.64	13.27	5.37	150	6	9.3
		A	30.04		18.64		156		9.0
	1:50	S	42.49	4.22	12.00	4.57	140	5	8.4
		A	38.27		16.57		145		8.9
	2:30	S	47.31	4.66	12.77	4.56	144	5	8.7
		A	42.65		17.33		149		9.1
5	12:15	S	39.20	3.09	10.40	3.77	135	7	9.9
		A	36.11		14.17		142		9.6
	12:45	S	41.60	3.67	8.38	4.33	138	11	9.3
		A	37.93		12.71		149		8.8
6	1:30	S	38.86	3.06	3.92	3.16	94	6	7.6
		A	35.80		7.08		100		7.2
	2:00	S	39.97	2.93	5.57	3.17	61	6	7.3
		A	37.04		8.74		67		6.9
7	1:15	S	42.76	3.17	11.14	6.82	149	7	9.4
		A	39.59		17.96		156		8.7
	1:40	S	50.47	4.15	13.59	4.26			9.1
		A	46.32		17.85				9.5

that an average of 4.6 volumes percent of oxygen was removed by these brains as the blood passed through them. The figures for the minute oxygen consumption per 100 grams of brain tissue (table 2) show a fairly wide variation among the different experiments; although the cerebral oxygen consumption in any one dog appears more or less constant from time to time, under the conditions of the experiment. The average value for the seven experiments is 10.9 cc. per 100 grams of brain per minute, a value that is in fairly close agreement with results

TABLE 2

DOG NO.	WEIGHT	BLOOD FLOW		PERFUSION PRESSURE	MIN. CONSUMPTION PER 100 GRAMS BRAIN		QO ₂
		cc./min.	Per 100 grams Brain/min.		Oxygen	Dextrose	
	<i>kgm.</i>				<i>cc.</i>	<i>mgm.</i>	
1	13.6	160	245	150	11.0	11	6.6
		170	260	150	10.5	9	6.3
		170	250	155	9.7	11	5.8
2	17.3	176	240	140	13.6	15	8.1
		142	192	150	10.7	13	6.4
		140	188	155	10.9	15	6.7
3	19.4	224	340	118	10.2	13	6.1
		204	310	118	11.3	15	6.8
4	18.6	184	240	120	12.9	14	7.7
		212	265	130	12.6	13	7.5
		208	270	125	12.6	12	7.5
5	13.6	260	330	118	12.1	23	7.2
		178	230	160	9.9	20	5.9
6	11.8	240	310	120	9.8	18	5.9
		220	280	130	9.1	16	5.5
7	12.3	96	135	85	9.3	10	5.6
		160	220	120	9.5		5.8
Averages.....					10.9	14	6.8

obtained by less direct means (13, 14, 15). Calculations of the Q_{O₂} values (cmm. oxygen consumed per mgm. of brain tissue per hr.) gives an average of 6.8.

During the passage of blood through the brain there was an average uptake of 7 mgm. per cent of dextrose. The average dextrose consumption per 100 grams of brain per minute was 14 mgm. The whole brain, in these experiments, used about 0.5 gram of dextrose per hour; and in order to keep the blood sugar level within normal limits, small amounts of sugar had to be added to the perfusing blood from time to time.

No evidence was obtained from these experiments to indicate lactic acid formation or utilization by the brain. The lactate content of the arterial and venous blood remained the same throughout an experiment.

DISCUSSION. The viability of the preparation could readily be ascertained by observing the eye reflexes. The pupillary and palpebral reflexes are more sensitive to oxygen lack than are certain of the vital centers, such as the cardiac, vasomotor and respiratory centers (16). Furthermore, the work of McFarland, Knehr and Behrens (17) indicates that ocular activity is a sensitive indicator of hypoxia of the brain. The blood supply to the eyeball was therefore left intact. Trial experiments demonstrated that the blood flow to the eye is too small, in comparison with the total cranial flow, to introduce a significant error in calculating the oxygen consumption figures for the brain.

An important question to be answered is whether the perfusion was limited to the brain tissue alone. At the conclusion of each experiment a dye was injected into the arterial blood flow to the brain. In no case did more than insigni-

TABLE 3

DOG NO.	O ₂ CONSUMPTION OF INTACT DOG	O ₂ CONSUMPTION OF BRAIN	PER CENT OF TOTAL CON- SUMED BY THE BRAIN
	<i>cc. per minute</i>	<i>cc. per minute</i>	
1	101	7.4	7.3
2	128	10.2	8.0
3	144	9.5	6.6
4	138	11.1	8.1
5	103	8.5	8.2
6	87	7.3	8.4
7	91	6.7	7.4
Average.....			7.7

nificant amounts of dye appear in tissues other than brain substance and the eyeball. The dye appeared in the brain tissue down to the lower end of the medulla. The brain was sectioned at this point and removed and weighed.

The figures for blood flow cannot be regarded as comparable to cerebral blood flow in the normal animal. Although the mechanical pump delivered pulsatile flow and the perfusion pressure was maintained at approximately the carotid pressure of the normal dog, the small arterio-venous oxygen differences, when compared with values reported in the literature, indicate that the blood flows were greater than normal.

The blood gas values of sinus blood and the venous outflow from the head were the same, a further indication that no significant blood flow to tissues other than the brain occurred. Preliminary perfusion experiments in which all the muscular branches of the carotid arteries were not ligated gave an apparent oxygen consumption of 20.5 cc. per 100 grams of brain, a figure nearly double the value obtained after ligation of the extra-cranial arteries.

With the limitations in mind of determining the respiratory quotient of tissues

by arterio-venous blood gas differences (19), the results of these experiments are perhaps noteworthy in that they agree closely with earlier observations on the R.Q. of the brain. Lennox (19) found an average brain R.Q. of 0.95 in human subjects and Himwich and Nahum (15) reported a value of 1.0 for dogs. In the sixteen observations of these experiments, the average brain R.Q. is 0.94 (excluding the first observation of expt. 7 which is inexplicably low).

Table 3 shows a comparison of the basal oxygen consumption of the intact dog with that of the brain.

The Q_{O_2} values are calculated on the basis of the wet weight of the brain. They may be converted to dry weight figures by multiplying by the factor 5 (18).

SUMMARY

A method of isolating the circulation to the dog's brain and a perfusion technique are described. The average minute oxygen consumption per 100 grams of brain in seven experiments, using the above technique, was 10.9 cc. giving a Q_{O_2} of 6.8. The average brain R.Q. was 0.94. Observations on dextrose and lactic acid metabolism by the brain are also recorded. These experiments indicate that approximately 50 per cent of the oxygen supplied to the head region is used by the brain and that the brain accounts for about 8 per cent of the total oxygen consumption of the body at rest.

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THE EFFECTIVENESS OF PLASMA, GELATIN AND SALINE TRANSFUSIONS IN PREVENTING SHOCK INDUCED BY LEG MUSCLE TRAUMA AND TOURNIQUETS¹

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In a study in which shock was produced in dogs by the release of tight leg tourniquets after a five hour period of constriction, it was observed that saline infusions and a single large plasma transfusion could prevent death in but a few of the cases (1). When, however, the same amount of plasma was divided into five equal doses, and given intermittently over the whole of the critical period, shock was prevented in all cases. These results led us to make a comparative study of the effectiveness of plasma, gelatin and saline in preventing shock following leg tourniquet release, and also that following leg muscle trauma. Both the single infusion and intermittent injections were employed for the administration of the plasma and plasma substitutes.

Gelatin has been used in the treatment of shock by several groups of investigators within recent years (2-9). Their reports indicate that its effectiveness in restoring a depleted plasma volume after massive hemorrhage or after burns is somewhat less than that of plasma, but greater than that of a salt or other crystalloidal solution. Since different lots of gelatin, even when prepared in identical fashion, may vary somewhat, care was taken to reduce to a minimum the number of different batches used. All experiments were done with gelatin from two different lots.

A. *Tourniquet shock.* As in the experiments published earlier (1), shock was produced in a series of dogs by the tight application, as high on the hips of both legs as possible, of a heavy walled rubber tubing. In the previous publication, through a typographical error, the diameter of this tubing was quoted as 120 mm. instead of the correct 12 mm. The tourniquets were released after a period of 5 hours. The symptoms shown by the control animals for this experiment were identical in all respects with those previously described (1). There was a marked swelling of the injured legs, intense hemoconcentration (from 38 per cent hematocrit to 71 per cent), and a plasma volume reduction of 49 per cent. The evidence seemed clear that the essential factor contributing to the initiation of the shock condition was the extreme local fluid loss into the injured legs.

A plasma transfusion of 25 cc. per kgm. body weight given either immediately after release of the constrictions, or later, ameliorated somewhat the extent of

¹ The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Princeton University.

² Upjohn Research Fellow.

the hemoconcentration, but failed to prevent shock in 9 of 12 dogs (1). Saline infusions were even less effective. When the same amount of plasma was divided into doses of 5 cc. per kgm. body weight each, and given at 0, 1, 2, 4 and 7 hours after tourniquet release, all signs of shock were prevented in all cases of a series of 7 dogs. The positive effect could not be correlated with hemoconcentration changes.

In the present experiments, 25 cc. per kgm. body weight of a 5 per cent gelatin solution, prepared in physiological salt solution with an oncotic pressure of 70 mm. Hg after sterilization², was divided into 5 equal doses for intermittent transfusions into a series of 14 dogs. Thirteen of the transfused animals of this series showed no signs of shock at any time, and were eating full rations at the end of 24 hours from the time of tourniquet release. The single failure survived for 36 hours, maintaining a good arterial pressure throughout the interval, and then succumbed to a respiratory infection.

The other 13 dogs were kept ten days. The legs showed no development of gangrenous areas, nor were any ill effects of the gelatin observed. So far as

TABLE 1

Average blood pressure, pulse, and blood concentration changes in 14 dogs protected against shock following release of leg tourniquets by an intermittent transfusion of gelatin

TIME	BLOOD PRESSURE	PULSE PER MINUTE	HEMATOCRIT	HEMOGLOBIN	SERUM PROTEIN
	<i>mm. Hg</i>		<i>per cent</i>	<i>grams per cent</i>	<i>grams per cent</i>
Initial.....	104	130	40.2	14.3	5.72
Tourniquet release.....	102	133	50.8	17.7	6.59
4 hrs. after release.....	104	147	60.2	21.8	6.39
7 hrs. after release.....	99	149	60.5	21.2	6.27
24 hrs. after release.....	96	138	54.2	17.8	5.72

could be judged by blood pressure, hematocrit and hemoglobin changes, the gelatin was just as effective as plasma when given intermittently over the 7 hour period. In fact, the hemoconcentration tended to be less extreme than with plasma transfusions (table 1). Serum protein concentrations, measured as serum specific gravity by the falling drop apparatus, showed a slight fall in the gelatin infused animals, rather than the rise which follows plasma treatment (1). Since it had been shown previously that a single large transfusion of heparinized plasma was ineffective in this type of shock we did not attempt to test the gelatin by the single transfusion method.

B. Leg muscle trauma. The type of leg muscle trauma employed was similar to that first used by Kendrick, Essex and Helmholtz (10), Best and Solandt (11) and Cullen and associates (12), as later modified by Gregersen and co-workers (13). The animal is placed under ether anesthesia, and 400-800 blows delivered to all faces of the thigh muscles of each leg with a 200 gram rawhide mallet. The legs are numbed and severely bruised, but the skin is unbroken and no

² We are indebted to Dr. John F. Norton and the Upjohn Company of Kalamazoo Michigan for generous supplies of specially prepared sterile gelatin.

bones are fractured. Trauma is continued until the mean arterial pressure in the femoral artery as determined by the needle puncture method (14), has been reduced to 60–70 mm. Hg. Ether is then discontinued, and the animal tied on its back on the animal board for 6 hours, after which, if still alive, he is returned to his cage. No food or water is given the animals which recover until after the 24th hour.

Of a control series of 37 dogs subjected to this form of muscle trauma, 33 died in shock and 4 recovered. The average life span was 5 hours (15). As has been

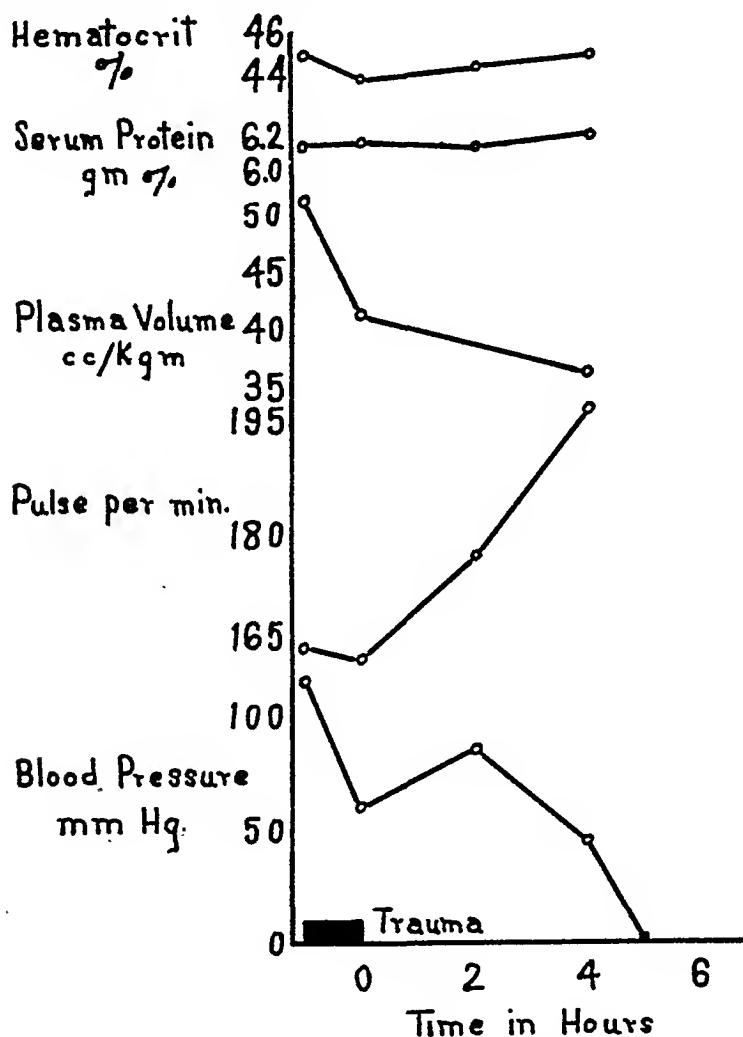


Fig. 1. Plasma volume changes after trauma

reported by others, the shock induced by this type of leg muscle trauma is not accompanied by consistent or significant changes in hematocrit, hemoglobin or serum protein. Blood changes shown by a representative animal are graphically presented in figure 1. The plasma volume, as measured by the dye T-1824 method (16), shows an average decline of 32 per cent before death. Detailed studies on the shock induced by leg muscle trauma will be reported later. We are concerned here solely with the effect of transfusions of plasma, gelatin and saline upon the prevention of shock induced by this procedure.

1. *Single transfusion of plasma.* In all plasma transfusion experiments, sterile heparinized plasma, obtained by rapid continuous bleeding of a large donor dog, was employed. Each of a group of 10 dogs, subjected to muscle traumatization, was given a single intravenous transfusion of 33 cc. per kgm. body weight. As with the controls, the blood pressure was reduced to below 70 mm. Hg before trauma was stopped. The pressure then showed a slow rise to 80-90 mm. Hg,

TABLE 2

Average blood pressure and pulse changes in traumatized dogs given plasma, gelatin and saline infusion

Blood pressure given in mm. Hg

	NO. OF DOGS	BODY WEIGHT kgm.	NO. DOGS SURVIVED	NO. DOGS DIED	INITIAL		AFTER-TRAUMA		PRE-TRANSFUSION		POST-TRANSFUSION		6 HOURS		24 HOURS		SURVIVAL TIME hours
					Blood pressure	Pulse per minute	Blood pressure	Pulse per minute	Blood pressure	Pulse per minute	Blood pressure	Pulse per minute	Blood pressure	Pulse per minute	Blood pressure	Pulse per minute	
Plasma, 33 cc./kgm., single transfusion	10	8.4	2	8	113 130	168 168	66 64	144 152	72 72	146 188	109 95	156 185	96 111	138 187	108 113	148 156	6
Plasma, 33 cc./kgm., intermittent transfusion	13	10.6	10	3	116 113	152 163	65 58	152 180									4
Gelatin, 33 cc./kgm., single transfusion	10	9.6	5	5	119 104	136 135	61 64	154 165	79 67	195 216	127 108	148 180	113 87	169 196	99	173	9
Gelatin, 33 cc./kgm., intermittent transfusion	10	9.6	6	4	118 121	161 159	64 58	157 158					107 95	167 188	107	158	10
1% salt solution, 33 cc./kgm., single transfusion	12	9.5	7	5	123 120	147 139	66 62	145 168	78 58	199 196	101 80	175 173	104 104	159 159	99	142	6
20% salt solution, 20 cc., single infusion	12	10.6	6	6	111 129	152 155	65 65	149 181	73 69	159 176	104 93	163 145	104 65	179 218	121	160	6
20% salt solution, 20 cc., intermittent infusion	12	10.6	6	6	122 123	165 143	62 66	145 153					105 83	154 163	120	130	8
15% salt solution, 30 cc., intermittent infusion	15	10.4	6	9	119 119	136 144	66 62	144 145					96 49	160 194	121	140	6

which level was maintained for a variable period. Transfusions were given only when the pressure level was again declining, and was below 85 mm. Hg (table 2). At least 1 hour was allowed from the time of the completion of the trauma. Of the 10 animals given the single plasma transfusion at this time, but 2 survived. Hematocrit and hemoglobin levels evidenced a moderate blood dilution, which was not rapidly lost (table 3). No correlation could be drawn between the

extent of this dilution and the symptoms of the animal. Serum protein concentrations were elevated.

2. *Intermittent transfusion of plasma.* An equivalent amount of plasma, i.e., 33 cc. per kgm. body weight, was divided into 5 equal doses, and given at 0, 1, 2, 4 and 7 hours after the trauma. Of 13 traumatized animals given this type of transfusion 10 showed no symptoms of shock and survived indefinitely, and 3 died in shock. Hemodilution changes were of the same general order as with the animals receiving the single plasma transfusion (table 3).

TABLE 3

Average blood concentration changes in traumatized dogs given plasma, gelatin and saline infusions

	NO. OF DOGS	INITIAL			½ HOUR AFTER TRANSFUSION			6 HOURS AFTER TRAUMA			24 HOURS AFTER TRAUMA		
		Hematocrit	Hemoglobin	Serum protein	Hematocrit	Hemoglobin	Serum protein	Hematocrit	Hemoglobin	Serum protein	Hematocrit	Hemoglobin	Serum protein
		per cent	gm. per cent	gm. per cent	per cent	gm. per cent	gm. per cent	per cent	gm. per cent	gm. per cent	per cent	gm. per cent	gm. per cent
Plasma, 33 cc./kgm., single transfusion.....	10	46.6	17.3	6.28	31.3	10.7	7.09	38.1	14.2	6.44			
Gelatin, 33 cc./kgm., single transfusion.....	10	47.0	16.5	5.93	28.4	10.3	4.96	36.4	13.0	4.90	42.3	14.9	6.09
Salt solution, 20%, 20 cc., single transfusion.....	12	47.5	17.3	6.12	44.5	16.6	5.86	43.9	17.2	6.03	39.5	13.8	5.11
Salt solution, 1%, 33 cc./kgm., single transfusion..	12	48.8	18.8	6.01	46.8	17.6	5.90	52.0	20.7	6.35	43.9	19.3	5.88
Plasma, 33 cc./kgm., intermittent transfusion.....	13	51.0	18.1	6.93	41.3	15.6	7.21	41.3	15.3	7.12	38.2	13.8	7.00
Gelatin, 33 cc./kgm., intermittent transfusion.....	10	49.2	17.0	6.67	41.8	15.0	6.13	42.1	15.4	5.71	50.7	14.9	6.21
Salt solution, 20%, 20 cc., intermittent transfusion..	12	46.3	16.1	6.02	41.4	14.4	5.78	39.0	14.6	5.80	36.0	13.2	5.47
Salt solution, 15%, 30 cc., intermittent transfusion..	15	49.6	17.7	6.50	47.5	17.4	6.40	44.4	16.4	6.25	40.4	13.8	5.62

The experiments demonstrate again that small intermittent transfusions distributed over a 7 hour interval are more efficacious in preventing shock, both after tourniquet release and after leg muscle trauma, than a single large transfusion.

3. *Single transfusion of gelatin.* A transfusion of 33 cc. per kgm. body weight of the 5 per cent gelatin solution was given at the time of the secondary blood pressure fall, and never less than 1 hour following the completion of the trauma. Of 10 dogs so transfused, 5 showed no signs of shock and survived, while 5 died in shock, with the survival span somewhat prolonged (table 2). Hemodilution was more marked than with the animals receiving plasma (table 3), but, again,

no correlation could be made between the extent of the dilution and the symptoms of the animal. Serum protein values showed a distinct decline rather than the rise which follows injection of plasma.

It should be noted that, in this experiment, the actual blood pressure level at the time the transfusion was begun (table 2) was lower in the animals which later succumbed than in those which recovered. In other words, the ineffectiveness of the gelatin infusion in 4 of the 10 animals might be correlated with the fact that the shock condition was more severe at the time the transfusion was started. Of the other 6, which showed blood pressure levels between 75 and 85 mm. Hg at the time the transfusion was started, shock was prevented in 5.

4. *Intermittent transfusion of gelatin.* Each of another series of 10 dogs was given the same total amounts of gelatin, 33 cc. per kgm. body weight, but divided into 5 equal doses, given at 0, 1, 2, 4 and 7 hours following conclusion of the trauma. Six of these animals exhibited no symptoms of shock, the other 4 died in shock, but the survival span was lengthened (table 2). The difference between the two modes of gelatin administration is not sufficiently clear cut in this experiment to permit a conclusion as to their relative merits.

5. *Single infusion of 1 per cent salt solution.* Since the gelatin was prepared in physiological saline, it seemed necessary to test the efficacy of saline alone in preventing shock. In the first of these experiments, 33 cc. per kgm. body weight of a 1 per cent salt solution was given as a single infusion at the time of the secondary blood pressure fall. Of a total of 12 dogs, 7 had arterial pressures between 75 and 85 mm. Hg at the time the infusion was begun and of these, 6 dogs lived indefinitely and 1 dog died after 12 hours. Of the other 5 animals which showed blood pressures below 75 mm. Hg when the infusion was begun, 1 dog recovered and 4 died in shock. Hence, for this whole series of 12 dogs, 7 recovered and 5 died in shock.

Hemodilution following the salt infusion was slight, and was not maintained (table 3). In fact, a tendency toward hemoconcentration followed within a few hours. Again, the survival of the animal could not be correlated with blood dilution.

6. *Single infusion of hypertonic salt solution.* Since the quantity of fluid administered is an important factor to be considered when comparing the value of blood substitutes on shock prevention, a series of experiments was performed in which small amounts of highly concentrated salt solution were used. These solutions were given intravenously at a rate not exceedingly 0.5-1.0 cc. per minute.

In the first of these experiments, 20 cc. of a 20 per cent salt solution were given at the time of the secondary pressure fall to 12 dogs. Six of these remained symptom free and survived, and the other 6 died in shock (table 2). The pre-injection pressure levels were of the same order in the animals which died and those which survived. The infusion caused an immediate hemodilution, which, however, was less marked and of shorter duration than that which followed use of either plasma or gelatin (table 3). Owing to the intense thirst aroused by the injection of the strong salt solution, water was allowed at the end of the

8th hour instead of waiting a full 24 hours as in the plasma and gelatin experiments. The ingestion of water did not seem to influence the results.

7. *Intermittent infusion of hypertonic salt solution, 20 per cent.* Twenty cubic centimeters of a 20 per cent salt solution was divided into 5 doses, and given intermittently over the first seven hours following trauma to 12 dogs. Six of these showed no signs of shock, and 6 died in the usual time interval. There was, therefore, no indication that the intermittent saline injection was more efficacious than the single infusion. Hemodilution was as large as that observed following either plasma or gelatin transfusions (table 3).

8. *Intermittent infusion of 30 cc. hypertonic salt solution, 15 per cent.* Fifteen traumatized dogs were given 5 intermittent injections of 6 cc. each of a 15 per cent salt solution over a 7 hour interval. Nine animals of this series died in shock, and 6 survived. This proved the least successful series. A possible explanation for the rather low survival rate may have been the deleterious effect of the high temperature and high humidity which prevailed in the laboratory at the time these experiments were performed.

DISCUSSION. In so far as the treatment of shock following release of leg constrictions is concerned, the efficacy of intermittent transfusions over single transfusions seems quite clear. Neither a single infusion of salt solution nor one of plasma can prevent shock (1). The same amount of plasma, divided into 5 equal doses, and given intermittently over a 7 hour period, is highly successful in protecting against shock. Likewise, a similar volume of a 5 per cent gelatin solution administered in similar fashion proved strikingly efficacious.

The shock following leg muscle trauma appears to be less responsive to transfusions. Once again the value of intermittent plasma transfusions over a single injection seems established, since in the former case 77 per cent of the animals survived, and in the latter but 20 per cent failed to show shock. The results obtained with gelatin are not so decisive however, for with both intermittent and single transfusions, the survival rate was of the order of 50 per cent. Since salt solution alone is also effective in about 50 per cent of the cases, it would be difficult, if not impossible, to differentiate between the gelatin and the salt effect in shock prevention in these experiments. Unlike the shock following leg constriction, that following leg muscle trauma seems less responsive to gelatin than to plasma transfusions.

The evidence seems clear that some degree of protection against shock which follows the type of muscle trauma employed is afforded by infusions of salt solution alone. A single large infusion seems as efficacious as repeated smaller injections, and a small volume of strongly hypertonic solution less valuable than a larger volume of isotonic salt solution. However, the experiments indicate that saline infusions are relatively ineffective in preventing shock when such infusions are started when the arterial pressure is very low and shock well advanced. On the other hand, saline infusions are apparently highly beneficial when given early and before the blood pressure has fallen markedly, i.e., before symptoms of shock appear. It seems not unlikely that large infusions of 1 per cent saline or 5 per cent gelatin, if given immediately following leg muscle

trauma, would effectively prevent shock in an even larger number of the traumatized dogs than the 50 per cent obtained in these experiments. This represents a positive effect since in a series of untreated controls but 4 of a total of 37 dogs survived.

SUMMARY AND CONCLUSION

1. Twenty five cubic centimeters per kilogram of 5 per cent gelatin in physiological salt solution given intermittently over a 7 hour period at the rate of 5 cc. per kgm. prevented shock in all of 14 dogs following release of leg tourniquets.

2. The intermittent method of transfusing small amounts of plasma is much more effective in preventing shock following leg muscle trauma, than is a single large transfusion. Ten of 13 dogs did not show symptoms of shock after trauma when given plasma intermittently whereas but 2 of 10 dogs survived when a single large transfusion was employed.

3. Both 5 per cent gelatin in physiological salt solution and salt alone either in isotonic or hypertonic solution given as a single injection or intermittently over 7 hours led to survival of approximately 50 per cent of the traumatized animals.

4. Saline infusions are relatively ineffective in preventing shock induced by leg muscle trauma when such infusions are started when the arterial pressure is low and shock well advanced. However, they are apparently highly beneficial when administered before shock symptoms have appeared.

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THE ECCENTRICITY OF STANDING AND ITS CAUSE¹

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The early investigators in the field of animal mechanics assumed that in the well-formed individual the vertical projection of the center of gravity of the body as a whole must lie in the midsagittal plane (Schäfer, 1900). Thus the human body in the *normal Stellung* of Braune and Fischer (1890) was represented as passively poised over the ankle joint in a strictly symmetrical posture. Although it is well recognized that gravity is the chief deforming force affecting the vertical alignment of man, the magnitude and disposition of the gravitational rotatory stresses have been little studied, and direct experimental confirmation of the symmetry of standing is difficult to find. We have observed that the vertical projection of the center of gravity tends to fall slightly to the left and behind the geometric center of the total supporting base (Hellebrandt and Braun, 1939; Hellebrandt and Fries, 1942). This posterosinistral stance eccentricity occurs with sufficient frequency to suggest that the deviation may have biological significance. We found it to be characteristic of about 80 per cent of the subjects thus far studied.

Anatomic and physiologic asymmetries are frequent in Nature. The population of the universe appears to consist mainly of right-handed individuals. The crown whorl of head hair, when viewed from above, has a clockwise twist in the great majority of persons (Newman, Freeman and Holzinger, 1937), but the fetal rotation of the gut is counter-clockwise in direction and duodenal hernias occur on the left in 76 per cent of the reported cases (Gushue-Taylor and Hayward, 1942). There is a preponderance of curvatures of the spine convex to the left (Kuhns, 1938), and thromboses of the left iliac vein (McMurrich, 1908). Inequality in breast size has been reported, the left commonly being the larger (Gray, 1942). There is usually some lateral torsion of the uterus from left to right (Schumann, 1936). This is exaggerated during gestation and may reach 70 degrees. Occasionally the torsion may be in the opposite direction but statistics show that it occurs from left to right in 80 per cent of cases (Stander, 1941). Searching for an explanation of stance asymmetry we postulated that this might be compensatory for a right-sided morphological preponderance associated with anterodextral functional limb preference (Hellebrandt and Fries, 1942). The object of this study was to determine whether there is significant antecedence in the volitional use, and difference in strength and size of the limbs of the two sides of the body.

METHODS. 1. *Limb preference.* The first observations of limb preference

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were made by the method of Irwin (1938). They were subsequently confirmed, using an electronic precedence indicator devised and constructed by Gilson (1943). The subject, isolated in a dark room, was seated with the hands or feet resting on telegraph keys. The operator, from an adjoining room, controlled a bank of four colored lights, suitably dimmed and adjusted in front of the subject at eye level. The test was of the complex dilemma type. Whenever a predetermined combination of lights flashed, the subject responded by lifting the hands or feet from the keys, replacing them immediately. Six light combinations were employed. The order in which they were administered was standardized by random choice, arrived at by throwing a die to each numeral of which one light combination was assigned. The series consisted of 83 light flashes containing 25 of the stimulating combinations in chance distribution. The series was administered in four consecutive bouts to estimate the preference of the upper and lower extremities, each thus responding 100 times. The keys upon which the hands or feet rested controlled thyatron tubes which operated electro-magnetic counters. If one limb was raised at least 10 microseconds before the other, its precedence was recorded on the appropriate counter.

2. *Strength.* Strength tests are notoriously unreliable. To minimize the influence of uncontrollable variations in response, four different tests were employed. The force of a maximum effort was measured with a standard grip dynamometer. Martin's strength test (1918) was modified and used to estimate resistance to a gradually increasing force. To expedite the administration of the test, equipment was constructed which allowed firm immobilization of the subject during the application of the pull. A padded sling was fixed at right angles to the part to be tested and attached to a spring scale. To permit accurate adjustment of the angle of pull, the scale was suspended at the appropriate level from a ridge pole which could be swung through an arc of 180 degrees. A steady, gradually increasing pull was supplied by a half horse power a.c. motor. At the instant the resisting muscular contraction was overcome, the motor was switched off and the scale read. The muscle groups studied were those which produce protraction and retraction of the shoulder girdle, flexion and extension of the forearm, hip joint extension, abduction and adduction, and knee joint extension. The maximum resisting force which could be summoned was measured.

Repeated contractions may give a better estimate of muscle strength than a single maximum positive or resistive effort. A comparative study was made of the strength of the third digit flexors of the right and left hands using the Mosso ergograph. The two sides were tested alternately. The rhythm of contraction was set with a metronome and kept constant. The total number of trials was also kept constant. The subject made 30 maximum efforts in 60 seconds. The initial load was 1 kgm. This was augmented by 0.5 kgm. increments at successive trials to 4, 4.5 or 5 kgm. depending upon the strength of the subject. A kymograph record of the fatigue curve was obtained and the total distance through which the load was lifted was measured.

A similar strength test was devised to appraise the staying power of the

lower extremities. The subjects pedaled the electrodynamic brake bicycle ergometer first with one and then with the opposite leg (Kelso and Hellebrandt, 1934). The free lower extremity was comfortably supported during the exercise. A clamp attached to a rigid vertical support stabilized the pelvis during the unilateral pedaling. Alternate subjects rode first with opposite legs. After a suitable recovery period the activity was repeated on the opposite leg. The subject rode against a heavy load at a comparatively slow rhythm. The rate of working ranged from 309 to 740 kgm.-m/min. at an average r.p.m. of 47. The majority selected 500 kgm.-m/min. Each trial was carried to exhaustion. To assist in the differentiation of spurious from physiologic end-points of fatigue, heart rate and blood pressure responses to the exercise were measured. Observations were made at 60 sec. intervals and continued through the whole of the exercise and recovery periods after the subject had been carefully stabilized in recumbency. The work was hard. Cramps of the leg muscles occurred frequently during or at the cessation of the exercise. Heart rates occasionally reached 200/min. The systolic blood pressure did not exceed 190 mm. Hg. The duration of exercise was the independent variable. If the cardiovascular responses to the shorter ride were comparable to those of the longer, it was concluded that an all-out effort had been made on both trials, and the difference in staying power was not of psychic origin.

3. *Limb volume.* To gauge morphologic differences in size which might account for stance eccentricity the volume of the appendages was measured, immersing the limbs in water at approximately skin temperature and estimating the quantity of fluid displaced. Since superiority in functional capacity may be associated with improvement in blood supply and an increase in the size of the peripheral vascular bed an estimate was also made of the fluid accumulating in each limb under the influence of the hydrostatic effect of gravity when allowed to act for a given period of time, 20 minutes, under strictly standardized conditions.

4. *Morphologic symmetry.* As a final test of symmetry, differences in the weight of the two sides of the body were observed in recumbency on a balance board which permits the location of the center of gravity in a plane passing from head to foot. The subject was carefully balanced on a movable platform suspended from knife edges with gentle traction applied to head, wrists and ankles through equalizing yokes. A counter-weight was used to nullify the distorting effect on the center of gravity produced by platform movement. A sight line was strung from head to foot in the plane of the knife edges and the subject was photographed from above. The distance between the sight line and selected anatomical points could then be measured.

RESULTS AND THEIR INTERPRETATION. The subjects of the investigation were normal young adult women, professional students in physical education who participate in a variety of symmetric and asymmetric activities. They were accustomed to the severe exercise of competitive athletics, were familiar with laboratory procedures and could be relied upon to put forth a maximum physical effort. Forty-seven women acted as the subjects for the limb preference

studies. The remaining observations were made on a group of 20, each being a subject for the series of 8 experiments. The results are summarized in table 1.

1. *Limb preference.* The limb preference data yielded 5400 responses for the hands and a similar number for the feet. The right hand responded first in 49 per cent of the total number of trials, the left in 51 per cent. The difference between the two is small. The same is true of the foot responses, except that the preference was reversed, and the difference between them was slightly greater, 53 and 47 per cent respectively. In the analysis of the group data a slight preponderance of choice fell to the right foot and to the contralateral or opposing hand, as though the preferred lower limb were being maintained in a position of readiness for action with the center of weight eccentric in a counterbalancing position.

TABLE 1

Mean preference, strength and size of the limbs and the difference between the right and left sides

	RIGHT			LEFT			DIFFERENCE		UNITS
	M	SD	V	M	SD	V	D	CR	
Arm preference...	47.50	28.58	60.17	52.80	27.69	52.44	5.30	0.91	% total trials
Leg preference....	52.59	32.85	62.46	47.69	32.43	68.00	4.90	0.73	% total trials
Mosso ergograph.	112.25	61.75	55.01	102.25	60.25	58.92	10.00	1.33	kgm.
Grip strength.....	33.76	4.75	6.39	27.22	4.10	6.85	6.54	4.43	kgm.
Martin test.....	445.53	80.61	18.09	431.47	74.52	17.27	14.06	0.53	lb.
Bicycle time.....	12.42	7.92	63.77	10.95	6.51	59.45	1.47	0.61	min.
Bicycle work.....	5686.67	3318.62	58.36	5001.50	2750.69	55.00	685.17	0.67	kgm.-m./min.
Arm volume.....	1.54	0.30	19.48	1.45	0.33	22.76	0.09	0.92	1
Leg volume.....	4.80	0.81	16.87	4.73	0.88	18.60	0.07	0.27	1
Arm edema.....	82.18	52.51	63.90	74.55	36.76	49.31	7.63	0.39	cc.
Leg edema.....	254.54	126.02	49.51	235.91	168.11	71.26	18.63	0.29	cc.

2. *Strength.* The right grip strength was significantly stronger than the left. This was true of all but one subject, who was left-handed. The average right grip was 34 kgm. strong as compared with 27 kgm. on the left. Thus the socially dominant hand was 24 per cent stronger than the contralateral extremity and 55 per cent of the total grip strength resided on that side. A total of 272 muscle groups were measured as to strength by the Martin test. The maximum resisting force developed on the two sides was practically identical, 50.80 per cent being distributed to the right and 49.20 per cent on the left. Thus the right side was only 3 per cent stronger than the left. Dawson (1935) states that the difference in strength between the two sides of the body is very small indeed. In 1918 Martin made a study of muscular strength and symmetry and concluded that the difference in the strength of the two sides of the body, when

measured by his method, was neither great enough nor sufficiently constant to involve serious error if the two sides were assumed to be equal.

The series of experiments with the Mosso ergograph yielded 262 kymographic fatigue curves. The right and left strength were again very nearly the same. The right middle digit was 10.08 per cent stronger than the same finger of the contralateral limb. The mean duration of the unilateral exercise of the bicycle ergometer on the right exceeded the endurance on the left by 13.5 per cent. The average total work done was well over 10,000 kgm.-m, 53.20 per cent being contributed by the right extremity and 46.80 per cent by the left.

3. *Limb volume.* The stronger right arm displaced more water than the left, the difference being 6 per cent. The legs were more nearly equal in size. The difference in the amount of water displaced was only 1.5 per cent greater on the right than on the left. These slight differences in limb volume were exaggerated when the stasis and edema of hydrostatic origin were measured. The findings suggest that greater strength may indeed be associated with an increased capacity of the vascular bed. The accumulation of five or six hundred cubic centimeters of fluid unequally distributed between the appendages of the two sides thus adds itself to the small differences in muscle mass associated with asymmetry of strength and limb preference. As seen from an examination of the critical ratios in the table, the only statistically significant difference between the right and left sides is in grip strength. This is perhaps to be expected because of the lack of homogeneity in this small group. The average weight of the subjects was 138.82 lb. but this important variable ranged from 106 to 173 so that great differences in strength and limb volume must follow. It is suggested that the sum of the various differences may reach proportions of significance to the mechanisms concerned with the maintenance of balance. It may be a matter of no great physiological moment to cope with one, whereas in the aggregate a group of small asymmetries may make themselves felt, especially if they fall persistently to the same side. Thus conceived, the data take on increased significance.

4. *Morphologic symmetry.* When the right and left sides of the body were compared with the subject in recumbency on the balance board, 85 per cent were observed to be heavier on the right side than on the left. Only one appeared to be strictly symmetrical. How much of this is due to differences in muscle mass and how much to inequalities in visceral weight is open to speculation. Lyman (1942) reports the weight of muscle in rats to be heavier on the right in 94 per cent of the experimental number. This raises the question as to whether human asymmetry is inherited from the quadruped.

If the right side of the body is, in general, stronger and heavier than the left, it remains of interest to consider why the average center of gravity is eccentric to the contralateral instead of the homolateral side. This may be a simple overcompensation phenomenon, acting in the anteroposterior as well as the transverse vertical plane. Standing is not a static phenomenon. It is, in reality, movement upon a stationary base. In stable and physiologically well poised individuals, postural sway may be virtually insensible but this is by no

means invariably true. That postural sway is inseparable from the upright stance has been abundantly recognized and extensively studied since the pioneer observations of Vierordt (1862). It follows that the center of gravity of the body as a whole must also shift incessantly during standing.

To suppose that the average position of the trajectory of the incessantly shifting center of weight will plumb over the exact center of the base is expecting much of the automatisms known to control posture. Undercompensation would be unbiological since biped standing, being a basic requisite for normal life and activity, is well protected. Overcompensations, on the contrary, are commonly observed in the normal functioning of all organ systems. Since the center of gravity falls well in front of the axis of rotation of the ankle joint, gravitational rotatory stresses keep the leg constantly in an unbalanced position. The stresses tending to tip the body forward are equilibrated by the antigravity extensors. If they overshoot their mark in efforts of protection, the average position of the observed center of gravity will fall behind the geometric center of the base. Similarly, the heavier right side of the body unbalances the stance in the transverse vertical plane, giving rise to a second rhythmic series of myotatic reflexes in muscles which respond in a manner destined to equilibrate the disturbing force. Each time a barrage of proprioceptive impulses elicited by stretch associated with postural sway impinge upon cord centers, the motor impulse called forth more than meets the unbalancing force and the average center of gravity falls off-center to the contralateral side.

SUMMARY AND CONCLUSIONS

A series of experiments designed to yield quantitative estimates of right and left-sided differences in size, strength and limb preference were performed on a small group of young adult women in an effort to elucidate the mechanism of the slight posterosinistral eccentricity of the vertical projection of the center of gravity of the body as a whole which characterizes the upright stance of 80 per cent of normal subjects. The evidence substantiates the following conclusions:

1. Morphologic and functional asymmetries occur in limb preference, volume and strength.
2. Although most of the observed asymmetries are too small to have statistical significance they constantly favor the right side.
3. It is suggested that in the aggregate these small dextral asymmetries in functional capacity associated with like differences in strength and size have the effect of a slightly eccentric counterweight on the incessantly shifting rotatory moments acting on the joints of the weight-bearing skeletal parts. The autonomous equilibrating muscular contractions called forth overcompensate for the force of this eccentric weight and the anteriorly unbalanced position of the leg by an amount great enough to result in a slight eccentricity in the location of the mean vertical projection of the center of weight in a position contralateral to the sum of the unequal stresses.

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ON THE CONSTITUTION OF PROTHROMBIN¹

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Prothrombin is recognized entirely by its activity. Little is known of its actual composition except that it is either a protein containing about 4 per cent carbohydrate (1), or is closely associated with such a protein. The possibility that prothrombin may be a complex like complement made up of several components has not been widely recognized since no experimental data in its support have heretofore been offered. It is the purpose of this paper to present a number of simple experimental findings which can best be co-ordinated and explained by postulating that prothrombin is composed of two essential and separable factors which appear to be combined with calcium.

Evidence of two components in prothrombin. When oxalated human plasma in an unstoppered container is placed in a refrigerator, the prothrombin as measured by the author's method (2) progressively decreases as illustrated in table 1. It has previously been shown (3) that the destruction of prothrombin can be hastened in human blood by heating the plasma to 38°C. and passing a current of air through it. It appears that oxidation is the cause of the diminution of prothrombin. This is supported by the finding that the disappearance of prothrombin can be prevented or retarded by a layer of carbon dioxide gas above the plasma.

The feeding of toxic sweet clover hay or its toxic principle, now known to be 3,3 methylene-bis-(4 hydroxy coumarin) (4), causes a profound reduction of the prothrombin of the blood. The typical course of the hypoprothrombinemia in dicumarol² poisoning is presented in table 2.

Since the prothrombin of the plasma is diminished both after storage and in dicumarol poisoning, it is to be expected that when the two types of plasma are mixed, the blended plasma should show a prothrombin content corresponding to the average of the two plasmas. Surprisingly this is not the case as experiment 1 demonstrates.

The fact that mixing the two plasmas, both of which showed a marked reduction of prothrombin, caused a restoration of the prothrombin above the normal level of human plasma, indicates that the diminution of prothrombin in the two types of plasma is not identical. The simplest explanation is that prothrombin is composed of two factors or components, one of which is labile in vitro and disappears when blood is stored, while the second becomes diminished when the animal is poisoned with dicumarol. For simplicity the first factor is designated as component A, and the second—component B.

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² The dicumarol used was kindly furnished by Eli Lilly and Company.

Component A. This factor is demonstrable by its ability to restore the prothrombin time of stored plasma. In testing for its presence or estimating its concentration, component B must first be removed. The latter is completely adsorbed by aluminum hydroxide, which does not remove component A. The test is carried out as follows: nine volumes of oxalated plasma are mixed with one volume of aluminum hydroxide cream. The mixture is incubated for 15

TABLE 1
Increase of prothrombin time in stored plasma

AGE OF PLASMA	PROTHROMBIN TIME (SECONDS) HUMAN PLASMAS							
	I	II	III*	IV	V	VI	VII	VIII†
<i>days</i>								
	12	11½	12	11½	11½	11½	11½	11½
1	14	16	15	12½	13½	12	12	12
2	16	17	17	14	15½	13	13½	12
3	20	19	21	14½	16	14½	15	12
4	22	21	23	16	19	17	15½	12
5	26	27	26	18	23	20	16	12½
6	31	33	31	19	27	27	22	12½

* The first three specimens show the rate of decrease of prothrombin usually observed during the winter months.

† Specimens 7 and 8 are from the same subject, but 8 was covered with a layer of carbon dioxide and stoppered.

TABLE 2
*Increase of prothrombin time after feeding dicumarol**

TIME	PROTHROMBIN TIME (SECONDS)	
	Rabbit	Dog
<i>days</i>		
	6 (100)†	6 (100)
1	12 (20)	11½ (20)
2	19½ (5)	20 (5)
3	44 (1½)	41 (1½)
4	110 (¾)	43 (1½)
5	195 (>½)	150 (½)
6	720 (>¼)	360 (>¼)

* Ten milligrams of dicumarol per kilogram of body weight was fed daily.

† The figures in parenthesis are the concentration of prothrombin in per cent of normal.

minutes at 37°C. and stirred frequently. The aluminum hydroxide is removed by centrifugation. To 3 volumes of stored human plasma is added 1 volume of the "alumina plasma" which is to be tested. For the determination, 0.1 cc. of the mixed plasma and 0.1 cc. of thromboplastin are transferred to a small test tube, and 0.1 cc. of 0.02 M calcium chloride forcefully blown in to obtain instantaneous mixture. The reaction is carried out at 37°C. and the coagulation time accurately measured with a stop watch.

It has been found that the content of component A in dog and rabbit blood is the same whether component B is removed physiologically by means of dicumarol or chemically with aluminum hydroxide as shown by experiment 2.

The concentration of component A appears to be much higher in dog and rabbit plasma than in human, but considerable fluctuation of factor A occurs in the latter. See experiment 3.

EXPERIMENT 1

The decrease of prothrombin in stored plasma and after feeding dicumarol

	PROTHROMBIN TIME	PROTHROMBIN CONCENTRATION†
	<i>seconds*</i>	
Human plasma I (stored 8 days).....	45	6
Dog plasma II (after feeding dicumarol).....	180	>1
Plasma I + Plasma II (equal volumes of each).....	10	<100

* The clotting time of 0.1 cc. of oxalated plasma mixed with 0.1 cc. of thromboplastin and 0.1 cc. of 0.02 M calcium chloride.

† In terms of per cent of normal on the basis of the prothrombin level in human plasma.

EXPERIMENT 2

Removal of component B from plasma with aluminum hydroxide and by feeding dicumarol

	PROTHROMBIN TIME
	<i>seconds</i>
Human plasma I (stored 5 days).....	24
Dog plasma II (treated with $\text{Al}(\text{OH})_3$).....	∞
Dog plasma III (after feeding dicumarol).....	180
0.3 cc. of plasma I + 0.1 cc. of plasma II.....	10
0.3 cc. of plasma I + 0.1 cc. of plasma III.....	10

EXPERIMENT 3

The concentration of component A in dog and in human plasma

	PROTHROMBIN TIME
	<i>seconds</i>
Human plasma I (stored 6 days).....	37
Dog plasma II (treated with $\text{Al}(\text{OH})_3$).....	∞
Human plasma III (treated with $\text{Al}(\text{OH})_3$).....	∞
0.3 cc. of plasma I + 0.1 cc. of plasma II.....	10
0.3 cc. of plasma I + 0.1 cc. of plasma III.....	25

It will be observed that the concentration of component A is much higher in dog than in human plasma. In fact the addition of dog or rabbit plasma, from which component B has been removed, to human plasma (either fresh or stored) reduces the prothrombin time to 9 or 10 seconds. This is shorter than that of normal plasma which is 11 to 12½ seconds. Whether this is to be interpreted that human plasma contains less than the optimum amount of component A in relation to its concentration of the B factor is a subject of further inquiry. The

rate of disappearance of component A from human plasma varies considerably. It is not possible at present to state whether this is due to a greater initial concentration or to variations in stabilizing factors of the plasma. It appears that there is a seasonal variation, but this requires further study.

Component A is destroyed by heat. If either alumina or dicumarol plasma is heated at 60°C. for 15 minutes, little active component A remains as shown by experiment 4.

Both component A and B are to a certain degree group specific. Thus, component A of chicken plasma does not reduce appreciably the prothrombin time

EXPERIMENT 4

The effect of heat on components A and B

	PROTHROMBIN TIME
	<i>seconds</i>
Human plasma (stored 9 days).....	50
Dog plasma II (treated with $\text{Al}(\text{OH})_3$).....	∞
0.3 cc. of plasma I + 0.1 cc. of plasma II.....	10
0.3 cc. of plasma I + 0.1 cc. of heated plasma II*.....	30
0.3 cc. of heated plasma I* + 0.1 cc. of plasma II.....	∞

* Incubated at 60°C. for 15 minutes.

EXPERIMENT 5

Group specificity of components A and B

	PROTHROMBIN TIME
	<i>seconds</i>
Human plasma I (stored 7 days).....	38
Chicken plasma II (treated with $\text{Al}(\text{OH})_3$).....	∞
0.3 cc. of plasma I + 0.1 cc. of plasma II.....	32*
Chicken plasma III (stored 6 days).....	42
Dog plasma IV (treated with $\text{Al}(\text{OH})_3$).....	∞
0.3 cc. of plasma III + 0.1 cc. of plasma IV.....	33†

* Rabbit brain thromboplastin was used. With chicken brain thromboplastin the prothrombin time was 105 seconds.

† Chicken thromboplastin was used. With rabbit brain thromboplastin the prothrombin time was 180 seconds.

of stored human plasma; and alumina rabbit plasma only slightly lowers the prothrombin time of stored chicken plasma (expt. 5).

The stability of component A in true or unmodified plasma. Chicken or goose plasma which can be kept liquid without the addition of a decalcifying agent shows little or no diminution of prothrombin when stored, whereas the oxalated or citrated plasma loses its prothrombin, or more accurately its component A, promptly, as shown in experiment 6.

This experiment clearly demonstrates that component A is stable when the plasma is in its native or unmodified state, whereas in oxalated or citrated plasma it is easily destroyed. This suggests that in blood the two components

are bound by calcium, and that they are thus protected against external factors such as oxidation.

Component B. As already stated, it is this factor which diminishes when an animal is fed dicumarol. Preliminary studies suggest that a deficiency of vitamin K likewise produces only a reduction of component B. Thus, in a patient with obstructive jaundice, a definite decrease in the latter was found.

Component B is destroyed by heating to 60°C. (see expt. 4). It is group specific as demonstrated in experiment 5. Aluminum hydroxide removes the component completely from oxalated plasma, but apparently does not adsorb it from true or unmodified plasma in which the calcium has not been removed and the prothrombin complex has been allowed to remain intact. The evidence for this was obtained by adding just sufficient heparin to rabbit blood to prevent

EXPERIMENT 6

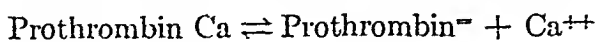
A comparison of the stability of prothrombin (or component A) in oxalated and in unmodified plasma

Unmodified chicken plasma....	0.1 cc.	Oxalated chicken plasma.....	0.1 cc.
Saline solution.....	0.1 cc.	Chicken thromboplastin.....	0.1 cc.
Chicken thromboplastin.....	0.1 cc.	Calcium chloride 0.02 M.....	0.1 cc.
Clotting time.....		Clotting time.....	11 sec.
		After 3 days storage*	
		12 sec.	18 sec.
		After 7 days storage	
		10½ sec.	32 sec.
Unmodified goose plasma.....	0.1 cc.	Oxalated goose plasma.....	0.1 cc.
Saline solution.....	0.1 cc.	Chicken thromboplastin.....	0.1 cc.
Chicken thromboplastin.....	0.1 cc.	Calcium oxalate 0.02 M.....	0.1 cc.
Clotting time.....		Clotting time.....	10 sec.
		After 4 days storage	
		10 sec.	32 sec.

* Blood was kept in small unstoppered test tubes coated with collodion. The temperature of storage was 10°C.

coagulation. Such plasma will on the addition of thromboplastin still coagulate readily. To 9 volumes of the heparinized plasma, 1 volume of aluminum hydroxide cream was added, and the mixture incubated at 37°C. for 15 minutes. As a control, a sample of the heparinized plasma was oxalated before treatment with aluminum hydroxide. The results are recorded in experiment 7.

The calcium factor. In a previous publication the author (5) postulated that prothrombin is a calcium compound which is slightly ionized.



This equilibrium is expressed by the equation:

$$\frac{\text{Prothrombin}^{\text{--}} \times \text{Ca}^{++}}{\text{Prothrombin Ca}} = K$$

Sodium oxalate or citrate inhibits coagulation by depressing the calcium ions. The action of sodium oxalate solution is relatively slow, probably because of the lag with which the equilibrium between soluble and insoluble calcium oxalate is established. It was found that sodium citrate acts promptly and therefore is better suited to study the depression of calcium ions and its effect on prothrombin.

Experimental. Blood was drawn directly into a test tube under a layer of mineral oil. The test tube was coated with collodion and the connecting glass tubing with paraffin. The test tube was immersed in ice, and carbon dioxide run through the apparatus just prior to the collection of the blood. By exercising great care in preventing the blood from coming in contact with air or glass surface, coagulation could be retarded 6 hours or more.

From the findings recorded in table 3, it can be seen that more citrate is needed to suppress the coagulation of rabbit blood than of human blood. This is to be

EXPERIMENT 7

The action of aluminum hydroxide on component B in decalcified and non-decalcified plasma

Heparinized rabbit plasma*	0.1 cc.	Heparinized rabbit plasma treated with $\text{Al}(\text{OH})_3$	0.1 cc.
Saline solution	0.1 cc.	Saline solution	0.1 cc.
Thromboplastin	0.1 cc.	Thromboplastin	0.1 cc.
Clotting time		Clotting time	16 sec.
Oxalated heparinized plasma†	0.1 cc.	Oxalated heparinized plasma treated with $\text{Al}(\text{OH})_3$	0.1 cc.
Thromboplastin	0.1 cc.	Thromboplastin	0.1 cc.
Calcium chloride 0.02 M	0.1 cc.	Calcium chloride 0.02 M	0.1 cc.
Clotting time		Clotting time	∞

* The blood contained 6 Toronto units (0.006 mgm.) of heparin per 5 cc.

† The blood contained 10 Toronto units (0.01 mgm.) of heparin per 5 cc.

expected since the author (6) has found by various methods that rabbit blood contains five times more prothrombin than does human blood. If it is assumed that only prothrombin Ca is active, i.e., is convertible to thrombin, one can conclude that prothrombin can be estimated at least roughly by titration of the plasma with sodium citrate. Thus, if 0.02 cc. of 0.1 M sodium citrate is added to 0.5 cc. of human plasma, the prothrombin time is increased to 15 seconds which according to the author's prothrombin curve indicates a 50 per cent reduction of the prothrombin. An inspection of the data of table 3 shows that if the calcium ion concentrations be plotted against the prothrombin times, a hyperbolic curve is obtained resembling the typical and familiar prothrombin curve. The exact mathematical treatment of the data will be reserved for a future publication.

It is interesting that Ransmeier and McLean (7) studying the effect of the calcium ion concentration on the coagulation time of citrated plasma likewise

obtained a rectangular hyperbola curve which corresponded to the empirical equation: $t = \frac{K}{Ca^{++-m}} + n$ (t = clotting time and K , m and n = constants).

The prothrombin curve, it will be recalled follows the equation $t = \frac{K}{c} + a$ (c = concentration of prothrombin; K and a = constants). On the assumption that the amount of active prothrombin is proportional to calcium ion concentration, the similarity of the two curves becomes apparent. Ransmeier and McLean did not control the thromboplastin, which conceivably can vary in dog and human blood; nevertheless their finding that human plasma requires a higher concentration of calcium ions (0.35 mM per liter of plasma) than dog plasma (0.28 mM) is entirely in accord with the present findings.

TABLE 3

The inhibition of coagulation (in the presence of excess thromboplastin) by sodium citrate

PERIOD OF INCUBATION	SODIUM CITRATE 0.1 M ADDED TO 5 CC. OF PLASMA										
	0	0.01 cc.	0.02 cc.	0.03 cc.	0.04 cc.	0.05 cc.	0.05 cc.	0.07 cc.	0.08 cc.	0.09 cc.	
	Clotting time (seconds)										
10 sec.	5½	6	6	6½	9	10	15	22	73	120	Rabbit plasma
11 min.	*	6	6	6½	8½	11	16	33	80	150	
3 min.		*	*	7	9	11	16	30	80	160	
10 min.				*	*	*	16	32	90	160	
10 sec.	11	12	15	36	75	210	480				Human
1 min.	10½	12	15	39	100	210	†				
3 min.	*	11½	15	30	95	220					
10 min.		*	15	32	80	190					

The clotting time was determined by mixing 0.1 cc. of the citrated plasma with 0.1 cc. of saline solution and 0.1 cc. of thromboplastin emulsion.

* Plasma clotted spontaneously.

† Only a few shreds of fibrin but no solid clot formed.

DISCUSSION. The data presented support the concept that prothrombin is a complex composed of three factors: component A, component B and calcium. By the removal of calcium the prothrombin is dissociated, but on the readdition of ionic calcium a resynthesis of active prothrombin immediately occurs. A diminution of any one of these three factors causes a decrease of the prothrombin as measured by the one-stage method. There is suggestive evidence that component A is somehow related to the oxidation and reduction system of the blood; whereas component B appears to be the body of the prothrombin complex, the factor which disappears in dicumarol poisoning and perhaps in vitamin K deficiency. Further work, however, is required before definite statements concerning these factors can be made.

The new concept is of theoretical importance, especially in emphasizing that biological agents may be complexes that are easily dissociated and resynthesized. It is to be remembered that complement which bears many resemblances to

prothrombin is composed of several well recognized components. The difficulty of isolating such a biological agent as a chemically pure substance is easily comprehended.

Several practical considerations arise from the hypothesis that prothrombin is a three-component complex. The first concerns the quantitative determination of prothrombin. Little is known concerning the quantitative relationship of component A to B, and therefore it seems hazardous to depend on results obtained by high dilution of plasma since one must assume that prothrombin which is dissociated in decalcified plasma is on recalcification resynthesized, i.e., components A, B and calcium are recombined. The use of plasma treated with aluminum hydroxide as a prothrombin-free medium may, as seen in the light of these new developments, lead in certain experiments to serious errors. The author in developing the prothrombin curves fortuitously usually employed human alumina plasma which is relatively low in component A. Fortunately, too, the alumina plasma was generally used as a diluent; and at high dilution of component B, the effect of component A is slight. Nevertheless, aluminum hydroxide-treated plasma can no longer be considered merely as being prothrombin-free—cognizance must be taken of its content of component A.

Views on the use of stored plasma for transfusion must be modified. Heretofore, such plasma was considered unsuitable for treating hypoprothrombinemia. Since stored plasma only loses component A and as only component B is depleted in dicumarol poisoning, the employment of stored plasma should be equally as effective as fresh plasma. This is of practical importance since patients receiving dicumarol may precipitously develop a hemorrhagic condition that demands an emergency transfusion.

With the realization that prothrombin is composed of several factors, a clearer and fuller understanding of the hypoprothrombinemias should be attained and a more concise classification should be possible. At present all the cases of clinical hypoprothrombinemia appear to be deficiencies of component B. Recently the author discovered a constant prothrombinopenia in an otherwise entirely normal young adult. The prothrombin remains at 45 per cent of normal and is not influenced by the administration of vitamin K. A decrease of component B was found to be responsible for the low prothrombin.

Although depletion of component A has been observed only *in vitro*, it is entirely probable that clinical hypoprothrombinemia due to lack of this factor may occur. Preliminary studies on chloroform poisoning in dogs have yielded results that indicate a temporary fall of both components.

SUMMARY

1. Experimental findings are presented which indicate that prothrombin is composed of calcium and two separable components designated A and B.

2. Component A disappears from oxalated plasma when stored in a refrigerator—it is presumably destroyed by oxidation. It is heat labile and to a certain degree group specific. In unmodified plasma, it does not diminish and therefore it can be concluded that factor A when present in the intact prothrombin complex is stable.

3. Component B disappears in the plasma of animals poisoned with dicumarol. It is heat labile and is completely removed from oxalated plasma by aluminum hydroxide. This adsorbent does not remove the factor from unmodified plasma, thus indicating that when it is combined in the prothrombin complex, it is not adsorbed.

4. Decalcifying agents inhibit the coagulation of the blood by depressing the calcium ion concentration of the system:

$$\frac{\text{Prothrombin}^- \times \text{Ca}^{++}}{\text{Prothrombin Ca}} = K$$

More sodium citrate is needed to suppress the coagulation of rabbit than of human blood. This is in accord with the finding that rabbit blood contains more prothrombin than is found in human blood.

5. The importance of the concept that prothrombin is a complex of several components is discussed in relation to *a*, the quantitative determination of prothrombin; *b*, the use of stored plasma for transfusion in the treatment of hypoprothrombinemia from dicumarol poisoning, and *c*, the classification and more exact understanding of clinical hypoprothrombinemia.

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THE EFFECT OF EPINEPHRINE UPON FROG RENAL HEMODYNAMICS IN THE INTACT ANIMAL¹

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One of the classical studies in the history of renal physiology is that of Richards and Schmidt (1) who by direct observation described the glomerular circulation of the frog and noted the effects of small amounts of epinephrine² upon it. More recently an indirect clearance method of following the renal blood flow in the intact animal has been introduced by Smith, Goldring and Chasis (2). The object of this present study is to attempt to validate the indirect clearance methods of renal function examination by comparing results obtained through their use with those noted directly by microscopic examination of the functioning kidney, especially with respect to the effects of small amounts of epinephrine on the renal circulation.

Richards and Schmidt exposed the kidneys of pithed frogs for microscopic examination by transmitted light and injected dilute solutions of epinephrine into the anterior abdominal vein. Following the injections the glomerular tuft increased in diameter, became crowded with cells, and the blood flow through its capillaries was slower. The results were such that they might be explained either by accelerated inflow of blood due to cardiac stimulation or by constriction of the efferent arteriole. The former explanation was eliminated by maintaining a constant blood flow through the kidney during the epinephrine injections (Richards, Barnwell and Bradley, 3). In the latter experiments the kidney was perfused through the aorta at a constant rate with whole blood or oxygenated Hamburger's solution after the gastrointestinal tract and spleen were excised, the renal circulation isolated, and the kidney exposed for direct microscopic examination. Addition of small amounts of epinephrine to the perfusate resulted in an increase in the size of the glomerular tuft and an increase in perfusion pressure which indicated efferent constriction. This conclusion was further substantiated by Hayman (4) who directly measured pressure within the glomerular capillaries of the living frog and found it to rise after epinephrine was introduced into the circulation in dosages similar to those which Richards and Schmidt found to cause an increase in the size of the glomerular tuft and decreased glomerular flow. Hence, by direct observation it was determined that small amounts of epinephrine caused constriction of the efferent arterioles with subsequent increase in intracapsular pressure and decrease in renal blood flow.

When the dose of epinephrine was increased Richards and Schmidt observed

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² Although epinephrine has been variously referred to as adrenalin and adrenin in the literature of renal physiology the former is used exclusively throughout this discussion.

that blood flow in the glomerulus as well as in its arteriole stopped, apparently due to constriction of the afferent arteriole. It was noted, however, that while stoppage may occur in one glomerulus, in another glomerulus close to it the blood may keep on flowing with unaltered rapidity.

METHODS. In these experiments large well nourished bullfrogs ranging in weight from 400 to 750 grams were used. Because of the difficulties involved in getting accurate urine collections from the bladder or cloaca the ureters were exteriorized and cannulated with several inches of fine silk catheter a day or more before the experiment. In some instances when a measurement of only arterial renal blood flow was desired the renal portal blood supply was tied off at the same time. Cautery was used and this operation was done with practically no loss of blood.

The standard clearance procedures outlined by Smith (5, 6) were used. The diodrast clearance at low plasma concentrations was used to measure the minimal renal plasma flow (Smith, Goldring and Chasis, 2). The creatinine clearance was used to measure the rate of glomerular filtration (Forster, 7). In a few instances intermittence of glomerular activity under massive doses of epinephrine was studied and the methods discussed in a previous paper were employed (Forster, 8).

In an animal which weighed 480 grams (G_3) the following procedure was used in the conduct of an experiment. A priming dose of 15 mgm. of diodrast and 200 mgm. of creatinine in 15 ml. saline solution was injected into the dorsal lymph sac and the bullfrog was placed in a battery jar half full of water. One hour later the frog was tied supine upon a holding board and prepared for infusion. The saline infusion fluid contained 15 mgm. per cent diodrast and 200 mgm. per cent creatinine and was introduced at a steady rate into the dorsal lymph sac by means of the dropping mercury technique. Fluid thus introduced into the lymph sac very quickly entered the blood stream due largely to the pumping action of a pair of lymph hearts at the posterior end of the lymph sac on either side of the urostyle. After considerable preliminary trial and error it was found that the plasma diodrast and creatinine concentrations could be kept quite constant by controlling the rate of infusion and the concentration of these substances in the perfusate. After two hours of infusion the first blood sample (3.5 ml.) was taken from the ventricle and the first of a series of 3 fifteen minute urine collections from the cannulated ureters was started. At the end of the third control period 0.01 mgm. epinephrine in 1 ml. saline solution was injected into the dorsal lymph sac and then 8 more 15 minute urine collections were made until the experiment was terminated. A blood sample was taken at the middle of the experiment and another at the end of the last urine collection.

Coagulation of blood was prevented by the use of dry heparin in the syringe and blood was centrifuged immediately upon being withdrawn. Blood proteins were precipitated by the cadmium method of Fujita and Iwataka (9). Diodrast concentrations in plasma and urine were determined by Smith's modification of Alpert's method (10), and creatinine by the method of Folin and Wu (11).

RESULTS. The effects of varying amounts of epinephrine on renal hemodynamics in 18 different bullfrogs were studied, each experiment involving an

average of 12 urine collection periods. The effect of a small dose is illustrated in figure 1. The injection of 0.01 mgm. of epinephrine after three fairly constant control periods resulted in a marked fall in the renal plasma flow (maximal diodrast clearance) and a corresponding rise in the filtration fraction ($\frac{\text{creatinine clearance}}{\text{diodrast clearance}}$) with the result that the rate of glomerular filtration (creatinine clearance) remained constant. The response to epinephrine administration was very rapid and recovery was obtained after 90 minutes.

The effect of a massive dose of epinephrine (0.1 mgm. or more) is characterized by an initial drop in the rate of glomerular filtration, the renal plasma flow and the filtration fraction. Within 15 to 30 minutes, however, the filtration fraction very rapidly increases and the filtration rate comes back to normal. The renal plasma flow remains low and does not increase until the epinephrine effect wears off and the filtration fraction drops. Tubular glucose reabsorption studies indicate that glomeruli close down in proportion to the initial drop in filtration rate.

The results indicate that epinephrine at normal levels exerts its effect on frog renal hemodynamics simply by causing constriction of the efferent glomerular arterioles. Only at high and probably abnormal levels does it affect the afferent arterioles causing constriction and stoppage of some glomerular activity.

DISCUSSION. These results obtained by the indirect methods of clearance analysis stand in complete agreement with those obtained in Richards' laboratory by direct observation of the functioning frog kidney. This, we think, constitutes a distinct link in the chain of evidence indicating that clearances provide reliable estimates of the various aspects of renal activity.

It is interesting to note that small amounts of epinephrine have exactly the same effect on both the amphibian and mammalian kidney. Chasis, Ranges, Goldring and Smith (12) have demonstrated in man that the renal blood flow appears to be controlled predominantly by the efferent glomerular arterioles. This has the effect of maintaining a filtration rate independent of the renal blood flow because variations in blood flow are accompanied by a corresponding inverse change in filtration pressure and filtration fraction which tends to maintain a constant filtration rate. Despite the fact that the frog maintains a constant filtration rate under the influence of small amounts of epinephrine, it must be remembered that afferent control is an important factor in maintaining water balance in these animals. Unlike mammals, variations in urine flow in the frog and other primitive vertebrates are accomplished by variations in the rate of glomerular filtration and in the number of functioning glomeruli. These alterations in glomerular activity are controlled largely by the afferent arterioles (Richards and Schmidt; 1, Forster, 7, 8; Friedlich, Holman and Forster, 13). Amphibians in and out of water are exposed variably to conditions requiring very rapid water elimination or extreme water conservation. Here the glomerulus assumes its primitive function and is the primary factor in regulation of water balance in contrast to the state in mammals where variations in urine flow are dependent entirely upon tubular reabsorptive activity.

The filtration fraction of the animal represented in figure 1 is quite low compared to that observed in mammals largely because this animal had its renal

portal blood supply intact during the experiment; hence, its maximal diodrast clearance represents not only the arterial blood which was delivered through the glomerulus but that venous fraction which supplied the tubules directly. The filtration fraction was considerably higher when the renal portal supply was removed before the experiment. Under the latter conditions 12 to 15 per cent of the blood delivered to the kidney is filtered in comparison with only 6 per cent when the portal supply is intact. The diodrast clearance at low plasma level (1 to 3 mgm. per cent) with the portal supply intact is about 700 ml. per kgm. per hr. and about 350 with the independent venous circulation removed. Appar-

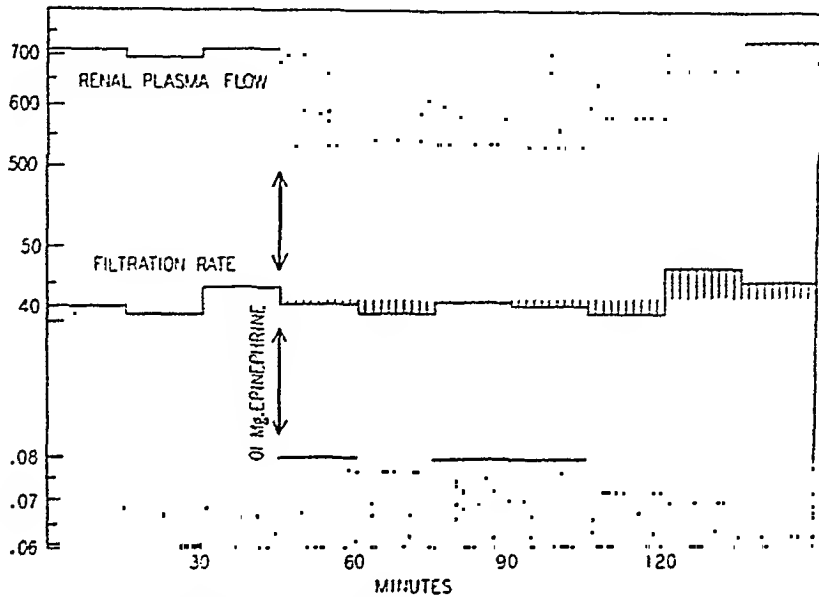


Fig. 1. A graphic illustration of the effect of 0.01 mgm. epinephrine on renal hemodynamics in a bullfrog (G_4) weighing 480 grams. The epinephrine was administered after 3 fifteen minute control periods and resulted in a marked drop in the renal plasma flow and a corresponding rise in the glomerular filtration fraction. The inverse relationship between plasma flow and filtration fraction indicates constriction of the efferent glomerular arterioles and results in maintaining a constant rate of glomerular filtration. This animal had its independent renal portal circulation intact which accounts for the relatively low filtration fraction. The renal plasma flow was calculated as the maximal diodrast clearance, and the filtration rate as the creatinine clearance. Renal plasma flow and filtration rate are expressed as milliliters per kilogram per hour and are both plotted on the same logarithmic scale.

ently about half the blood coming to the bullfrog kidney is arterial and half venous. Diodrast is excreted considerably more efficiently than phenol red by the frog. The maximal phenol red clearance obtained with the portal supply intact was 185 ml. per kgm. per hr. at a plasma concentration of 0.5 mgm. per cent (Forster, 14).

SUMMARY

The effects of epinephrine on frog renal hemodynamics as studied by the indirect clearance methods are precisely the same as those noted by direct observation of the functioning kidney. This is interpreted as constituting further

evidence for the reliability of clearance methods in evaluating the various aspects of renal activity.

The administration of small amounts of epinephrine into the circulation results in decreasing the renal blood flow, increasing the filtration fraction and, hence, maintaining a constant rate of glomerular filtration. This effect is obtained by causing constriction of the efferent glomerular arteriole.

Constriction of the afferent arteriole accompanied by decreased renal blood flow, filtration fraction, filtration rate and number of functioning glomeruli results from the administration of massive doses of epinephrine.

In the normal frog, unlike mammals, afferent control of the glomerular circulation plays an important rôle in the regulation of glomerular activity. Variations in the rate of water elimination result primarily from alterations in rate of glomerular filtration and only secondarily from variations in the rate of tubular water reabsorption.

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BLOOD PRESSURE RESPONSES OF DOGS TO VITAMIN A AND VITAMIN D₂¹

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Certain findings in the literature appear to indicate that large doses of vitamins A and D have a striking effect on blood pressure. It was felt that this needed reinvestigation.

Vitamin A. The use of vitamin A as an anti-hypertensive agent was first suggested by Govea-Pena and Villaverde (1) who claimed a favorable response of hypertensive patients to large doses of vitamin A. This action was apparently confirmed on dogs with experimental renal hypertension by Wakerlin et al. (2) who reported striking reductions in blood pressure in 3 hypertensive dogs beginning 2 weeks after daily oral administration of 200,000 units of vitamin A in 1 cc. of sesame oil. This reduction persisted for 6 months; the dosage in the last 3 months having been doubled. Sesame oil itself had no such effect on 2 dogs.

We repeated the study using 10 hypertensive and 1 normotensive dog. The hypertensive group included 5 rendered hypertensive by partial occlusion of one or both renal arteries, 3 in which hypertension followed temporary complete occlusion of both renal arteries for about an hour, and 2 in which the hypertension was spontaneous (cf 3). Hypertension in the dogs in the two 1st groups was of 100 to 850 days' duration. A control period of from 33 to 77 days was established in all dogs before instituting vitamin A therapy. Vitamin A dissolved in fish oil² was fed in doses of 400,000 units daily for periods of 45 days. In two of the dogs rendered hypertensive by partial renal artery constriction, a second course of therapy was instituted 56 days after the first. Vitamin A was given for 55 days in the same dosage as before, but dissolved in sesame oil instead of fish oil.³ Blood pressures were recorded with the Hamilton needle manometer as previously described (3). Adequate absorption was tested by determination of the plasma level three weeks after the beginning of treatment according to the method of Carr and Price. A 200-fold increase in the plasma level of vitamin A was found at this time.

There was in no instance a significant change in blood pressure during the first 3 weeks of vitamin A administration. During the last 3 or 4 weeks no significant change was observed in 8 of the 10 dogs used. A drop in diastolic pressure

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² This material, kindly supplied by Abbott Laboratories, contained 100,000 units per cubic centimeter of fish oil.

³ This material was supplied to us by the kindness of Dr. G. Wakerlin. It came from the same supply that he had used for his first study.

occurred in the remaining two. One of these (X-56), a dog with Goldblatt hypertension, which received two courses of treatment, showed drops during this time averaging 26 mm. Hg and 16 mm. Hg below the average diastolic pressure in the control period. These reductions persisted for 25 and 26 days after vitamin A administration was stopped. The second dog, a spontaneously hypertensive animal, showed a drop in diastolic pressure averaging 13 mm. Hg during the second half of the therapeutic period. No toxic symptoms were observed in any of the dogs during or after vitamin A administration.

Our results, therefore, fail to confirm the observation previously reported that vitamin A consistently lowers the blood pressure of hypertensive dogs, but do show that such a response, mild in degree, may be individually encountered. This is in accord with the more recent results of Wakerlin et al. (4) who found no drop in blood pressure in a second group of hypertensive dogs treated with vitamin A.

Whether this occasional depressor action is due to vitamin A itself or to some other material contained in the concentrate, as suggested by the observations of Grollman and Harrison (5), cannot be stated.

An unexpected result obtained in our experiments was the occurrence of a significant rise of the blood pressure after vitamin A medication had been stopped in 7 of the 8 dogs not showing a depressor response during vitamin A treatment. These rises, averaging for the diastolic pressure 13, 16, 17, 21, 23 and 25 mm. Hg above the pre-treatment level in the hypertensive dogs, and 17 mm. Hg in the normotensive dog, began from 14 to 20 days after treatment and persisted for from 27 to 80 days. No rise occurred in the dog whose blood pressure was reduced both times with vitamin A therapy. This post-therapy hypertensive effect would of course be exactly contrary to the expected vaso-depressor effect for which vitamin A therapy was contemplated, and is a contra-indication for the use of vitamin A therapeutically.

It might be argued that in most dogs homeostatic mechanisms powerful enough to counteract the vasodepressor effect are brought about by the ingested material, and that these mechanisms persist unbalanced after therapy is ended. Whether or not this view is valid, does not alter the fact that the vitamin A concentrates used do not lower blood pressure in experimental hypertension except on occasion and then only to a slight degree. This latter possibility is offset by the post-treatment vasopressor reaction which occurs when vasodepression fails to occur during the therapy.

Vitamin D₂. Appelrot (6) first reported that feeding dogs for periods of 15 to 25 days with large quantities of vitamin D (vigantol, 70 to 90 micrograms/kilo body weight/day) produced a hypertension in normotensive dogs. This result was confirmed by Handovsky (7) and Goormaghtigh and Handovsky (8), using oral vitamin D₂ in dogs. Hypertension was reported to begin in a few days when doses of about 100 to 700 micrograms per kilo of body weight per day were used. However, when subacute lethal doses were used (13 to 20 mgm. per kilo body weight per day), hypotension resulted. Reed et al. (9) recently repeated these studies and were unable to obtain any elevation of blood pressure in rats following

oral administration of vitamin D₂ in doses sufficiently large to induce severe reactions such as loss of weight. They state further that they have not been able to produce hypertension in dogs or man with various forms of vitamin D.

In this study we used 10 dogs, 5 dogs with experimental renal hypertension, 3 dogs with spontaneous hypertension and 2 normotensive dogs. In the spontaneously hypertensive dogs, vitamin D₂⁴ dissolved in propylene glycol was administered for 31 days subcutaneously in doses of 40,000 units U.S.P. per day, in the other dogs vitamin D₂⁴ by mouth, 1 mgm. per day, was used (400,000 units U.S.P. per day).

In all but three dogs no significant blood pressure change was observed during or after vitamin D administration. In the three exceptions, significant blood pressure elevation was encountered. In the normotensive dog (Y-39) in which the carotid sinus had previously been denervated, a rise of blood pressure averaging 24 and 16 mm. Hg respectively for systolic and diastolic pressure was noted for the first fifteen days of vitamin D administration, which brought the diastolic pressure up to the lower limits of hypertension. This rise disappeared during the later period of vitamin D medication.

In one of the spontaneously hypertensive dogs (Y-231) the rise in pressure was most marked in the first fifteen days following termination of vitamin D administration. At this time the systolic/diastolic pressures had risen on the average 19/18 mm. Hg above the average control level.

The greatest and most persistent rise occurred in one of the renal hypertensive dogs (Y-84). It amounted on the average to 26/27 mm. Hg during the period of vitamin D administration and was even higher than this during the 15 days after vitamin D administration, amounting to 27/35 mm. Hg above the average control level.

It would appear, therefore, that vitamin D does not usually have a pressor effect in dogs. On occasion, however, it may produce a moderate blood pressure rise, even in animals already spontaneously hypertensive, or rendered so by partial renal artery occlusion. Such individual variation as occurs with vitamin D we have found to be the case with another steroid, desoxycorticosterone acetate (10), except that the pressor effect with the latter is more pronounced and occurs more consistently. The variability in response which we obtained with vitamin D₂ may account for the apparently contradictory reports in the literature. It would, however, be erroneous to leave the impression that vitamin D₂ is a consistent and powerful vasopressor substance. Our results suggest rather that it has this tendency, but only to a slight degree.

SUMMARY

1. We cannot confirm the observations that:
 - a. Vitamin A in large quantities lowers the blood pressure of hypertensive dogs.
 - b. Vitamin D₂ in large quantities raises the blood pressure in normotensive dogs.

⁴ Supplied through the courtesy of Winthrop and Company.

2. However, we have found that on occasion a dog will show:
 - a. A slight lowering of an elevated blood pressure with vitamin A concentrate in fish oil or sesame oil, and
 - b. A moderate rise in blood pressure with vitamin D₂.
3. Attention is drawn to the frequent occurrence of a moderate rise in blood pressure of fairly long duration which occurs after a latent period following cessation of vitamin A concentrate therapy.

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THE TRANSFER OF ANDROGENS IN PARABIOTIC RATS¹

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The transfer of hormones in parabiotic rats had been reported by Martins (1), who found that the estrogen produced by the stimulated ovaries of a normal rat in parabiotic union (Coelioanastomosis) with an ovariectomized female was transferred to the latter. Hill (2, 3) confirmed this, using pairs in which the body cavities were not connected. He also presented evidence which indicated that there was a transfer of the corpus luteum hormone from a pregnant rat to its non-pregnant partner. Kallas (4, 5) observed a precocious development of the ovaries in normal immature female rats in parabiosis with castrates, which he attributed to the passage of gonadotropic hormone from the castrated to the normal animal. Biddulph, Meyer and Gumbreck (6) found that relatively large quantities of estradiol or diethylstilbestrol must be injected into one partner of castrated female-female parabiotic rats before detectable amounts cross to the uninjected parabiont. In view of these experimental data it became of interest to determine the amount of androgen necessary to inject into one partner of castrated male-male parabiotic rats to obtain evidence of transfer of the hormone to the other.

MATERIALS AND METHODS. This study includes observations made on 55 castrated male-male parabiotic rats, 27 single castrated male rats treated with varying amounts of testosterone propionate,² 7 untreated single castrated male rats, and 8 single normal male rats.

The operative procedure used in uniting the animals was that described by Bunster and Meyer (7), except that metal clips were used instead of silk sutures in closing the skin incisions. Ether anesthesia was used, and all operations were performed under sterile conditions.

Littermate rats weighing 70 grams or more were united in parabiosis at 31 to 33 days of age, at which time both partners were castrated. The right partners were injected subcutaneously with varying amounts of testosterone propionate within a few hours after the operation. The daily dose of testosterone propionate was in 0.05 cc. of corn oil. The injections were made once daily for 10 days, and the rats were autopsied on the eleventh day following parabiotic union. The seminal vesicles and prostates of both partners were dissected free of other tissues and were weighed to the nearest milligram.

The single animals used for assaying the testosterone propionate were castrated rats of the same age and body weight as those in parabiosis. They were castrated at the same age, and were injected for the same period of time as were the

¹ This work was supported by a grant from the Wisconsin Alumni Research Foundation.

² We wish to thank Dr. Erwin Schwenk of the Schering Corporation for the testosterone propionate.

parabiotic rats. The daily amount of hormone was in 0.05 cc. of corn oil and the injections were made subcutaneously.

The following groups of rats were used as controls: 1, single normals; 2, single castrates, and 3, uninjected castrates in parabiosis. These rats were of the same age and body weight as the experimental animals, and were autopsied at 41 to 43 days of age.

RESULTS AND DISCUSSION. Table 1 shows the weights of the accessory glands of the castrated parabiotic rats following the injections of testosterone propionate into the right partner. The data are unselected and include all the animals that were placed on the experiment.

It is seen that there was no stimulation of either seminal vesicles or prostates in the left partner when 15, 25 or 150 gamma of testosterone propionate were injected into the right partner. The accessory organs of the injected partners in

TABLE 1

Weight of seminal vesicles and prostates of castrated parabiotic rats, one partner given testosterone propionate

NO. OF PAIRS	DAILY DOSE (γ)	LEFT-HAND PARTNER		RIGHT-HAND PARTNER*	
		Seminal vesicle weight \pm S.E.	Prostate weight \pm S.E.	Seminal vesicle weight \pm S.E.	Prostate weight \pm S.E.
		mgm.	mgm.	mgm.	mgm.
7		9.1 \pm 0.61	33.5 \pm 2.00	9.5 \pm 1.00	32.5 \pm 4.40
5	15	7.5 \pm 0.90	21.4 \pm 1.64	42.3 \pm 5.83	106.5 \pm 10.84
6	25	7.1 \pm 0.65	27.1 \pm 2.00	75.9 \pm 6.68	157.4 \pm 10.81
6	150	9.3 \pm 0.42	30.0 \pm 2.91	239.3 \pm 26.90	357.6 \pm 13.96
6	300	12.6 \pm 0.78	55.2 \pm 3.42	304.9 \pm 14.30	404.5 \pm 22.60
6	400	13.6 \pm 1.76	45.0 \pm 4.45	379.6 \pm 21.79	491.3 \pm 21.55
6	500	10.1 \pm 0.92	37.1 \pm 3.63	308.8 \pm 37.30	398.3 \pm 28.00
7	750	14.6 \pm 2.00	48.2 \pm 6.40	382.6 \pm 44.30	425.3 \pm 33.10
7	1,000	20.7 \pm 4.20	73.1 \pm 9.24	354.1 \pm 23.18	442.5 \pm 19.15

* Injections were made into the right-hand partner.

all three groups were heavier than those of normal animals of the same age. (See table 2.)

Stimulation of the male accessory organs of the untreated partner was obtained in the parabiotic rats in which the injected partner received 300 gamma or more of testosterone propionate. The mean weight of the seminal vesicles and prostates of the untreated partners of those pairs receiving 300 gamma was approximately the same as that of single animals receiving 6 gamma of testosterone propionate. (See table 2.) This indicates that the equivalent of approximately 6 gamma of testosterone propionate per day crossed from the injected to the uninjected partner under these circumstances. Biddulph, Meyer and Gumbreck (6) using castrated female-female parabiotic rats found that 1 gamma of estradiol or diethylstilbesterol had to be injected into one parabiont before the equivalent of 0.0125 gamma of estradiol or 0.025 gamma of diethylstilbesterol crossed to the uninjected rat. Their data show that a dose equivalent to 80

times the minimum stimulating dose of estradiol or 40 times the minimum stimulating dose of diethylstilbestrol were required to give evidence of transfer in female-female parabiotic rats. From the data presented in tables 1 and 2 it can be seen that 50 times the minimum stimulating dose of androgen for single castrated male rats was required before there was evidence of transfer from one partner to the other.

We are unable to explain adequately why such large quantities of gonadal hormones must be injected into one parabiont before detectable amounts can be demonstrated in the uninjected partner. Since Kallas (4) and others have shown that castration of a parabiont causes great increase in the size of the gonads of the unoperated partner, it is logical to believe that gonadotropic hormones are more readily transferred across the anastomosed tissue. It is to be emphasized, however, that the source of the hormones is the pituitary gland, that they are water soluble and are secreted directly into the blood stream.

TABLE 2

*Weight of seminal vesicles and prostates of castrated male rats given testosterone propionate**

NUMBER OF ANIMALS	DAILY DOSE (γ)	MEAN SEMINAL VESICLE WEIGHT \pm S.E.	MEAN PROSTATE WEIGHT \pm S.E.
		<i>mgm.</i>	<i>mgm.</i>
7		10.4 \pm 1.50	32.6 \pm 2.56
5	2	10.7 \pm 0.49	33.8 \pm 2.49
5	4	10.3 \pm 1.06	43.1 \pm 2.43
5	6	12.4 \pm 1.12	56.4 \pm 4.71
6	8	24.7 \pm 3.10	71.6 \pm 4.58
6	10	24.4 \pm 1.91	81.8 \pm 4.33

* The mean seminal vesicle and prostate weights of 8 single normal rats were 20.1 \pm 2.57 and 89.6 \pm 9.78 mgm. respectively.

The accessory organs of the injected partners showed an increase in weight with each increase in dosage of the testosterone propionate to and including 400 gamma, beyond which an increase in the quantity given did not produce any consistent increase in weight. The prostates and seminal vesicles of the uninjected partners of those pairs receiving 15, 25 and 150 gamma per day did not show any increase in weight over control pairs; those of pairs given 300, 400, 500 and 750 gamma per day were slightly heavier and the heaviest were obtained in those pairs in which 1000 gamma per day was given. The weights of the seminal vesicles and prostates of the uninjected rats of this latter group were approximately the same as those found in single castrate animals injected with 8 gamma of testosterone propionate per day, or the same as those in normal rats of the same age and weight. This indicates that the equivalent of approximately 8 gamma per day crossed from the injected to the uninjected partner. Hertz and Meyer (8) found that it was necessary to inject 15 gamma of testosterone propionate per day into the castrate male partner of male-female parabiotic rats to prevent ovarian hypertrophy of the normal female. As the data in table 1 show, at least 20 times this quantity of the androgen must be injected into one parabiont before there is any evidence of transfer to the other.

SUMMARY

One partner of castrated male-male parabiotic rats was injected with daily doses of testosterone propionate varying between 15 and 1000 gamma. Evidence of transfer of the androgen from the injected to the uninjected parabiont was found when the daily dose was 300 gamma or more. At this dose level approximately the equivalent of 8 gamma of testosterone propionate crossed to the uninjected parabiont. Seminal vesicles and prostate glands equal in weight to those of single normal rats were obtained in uninjected parabionts when 1000 gamma of testosterone propionate were injected into the other partner.

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EFFECTS OF ANESTHETIC DOSAGE OF PENTOBARBITAL SODIUM ON RENAL FUNCTION AND BLOOD PRESSURE IN DOGS

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Since many procedures in physiology and experimental surgery require the use of anesthesia, it is important to recognize the deviations from the normal which the anesthetic may produce. Pentobarbital sodium is widely used for experimental anesthesia but, as Mylon, Winternitz and de Sütö-Nagy (1943) point out, certain side effects of the anesthesia may mimic some phases of experimental shock. They note among these untoward effects suppression of renal function and lowering of blood pressure, and consider that, "while there are undoubtedly individual variations in the response to nembutal," i.e., sodium pentobarbital, "on the part of animals, the major factors are the size of the dose and the method of its administration." It was their experience that the use of a 25 mgm. per kilo intravenous dose of the drug was more satisfactory than a dose of 30 mgm. per kilo because it was less toxic. It seems likely that intraperitoneal administration would be still less depressing than intravenous use; the peak of its concentration in the blood would be reached more slowly than with intravenous dosage, while the direct entry of some of the drug into the portal blood would result in its early destruction in the liver. This is the reasoning behind the practice in this laboratory of anesthetizing dogs by intraperitoneal injection of a 2.5 per cent aqueous solution of pentobarbital sodium (Lilly) in a dosage of 30 mgm. per kilo body weight. That the anesthesia thus induced is not commonly associated with evidence of toxic depression is shown by the observations of renal function and arterial pressure which form the basis of this report.

METHODS. The observations of renal function include the measurement of plasma diodrast and inulin clearances, of maximum tubular capacity for diodrast secretion (Tm_D) and rate of urine flow and were made by methods described in detail in previous studies (Corcoran and Page, 1943; Corcoran, Taylor and Page, 1943). The effects of anesthesia are shown by contrasting observations made during anesthesia with those obtained in the same dogs when conscious and when training had accustomed them to the necessary procedures. The record of arterial pressure was obtained from a damped mercury manometer by femoral arterial puncture with a 20 gauge needle when conscious, and by exposure and cannulation of the femoral artery when anesthetized. During anesthesia, the observations begin at about 45 minutes after injection of the anesthetic and continue for from 30 to 50 minutes.

The effect of anesthesia on blood pressure was examined statistically by comparison of 150 observations of femoral arterial pressure in 145 normal dogs

under anesthesia with values derived from 585 observations of femoral arterial pressure in 27 normotensive dogs. To make the data comparable, it is necessary to exclude from the observations of blood pressure in the conscious state those temporary nervous influences which disturb it and which would not operate under anesthesia. The dogs on which the observations were made were for this reason carefully trained to the procedure and in each of these the average of the recorded pressures was calculated. This average was taken as the resting arterial pressure of that animal and the mean and standard deviations obtained from these 27 values were used for comparison with the levels observed in other dogs under anesthesia. It should be noted that the dogs in which the observations were made in the conscious state were not entirely normal, since unilateral nephrectomy and contralateral subcutaneous explantation of the kidney (Page and Corcoran, 1940) had been done before the observations were made. That these operations do not significantly influence arterial pressure is shown by the mean pressure of these dogs, viz., 129 mm. by mercury manometer, a value nearly equal to that of 127 mm. Hg dirotic femoral arterial pressure optically recorded in a group of normal dogs by Hamilton, Pund, Slaughter, Simpson, Colson, Coleman and Bateman (1939). The group is therefore normal as regards arterial pressure. Indeed, any effect of the operations on arterial pressure would probably have been an increase due to altered renal hemodynamics and initiation of mild renal hypertension. In such a case, the difference between normal dogs and those under anesthesia would be still greater than that we describe. The use of data from uninephrectomized dogs with unilateral renal explants is therefore justifiable for the purpose of the comparison.

The observations of arterial pressure are summarized in table 3 by methods suggested by Bradford-Hill (1937). Standard deviation (σ) of group I, the normotensive dogs, was calculated from the average arterial pressures of these 27 dogs as $\sqrt{\frac{\Sigma}{n}} \times \sqrt{\frac{n}{n-1}}$, where Σ is the sum of the squares of differences from the mean and N the number of observations. In group II was calculated $\sqrt{\frac{\Sigma}{n}}$. The standard deviation of the mean was taken as σ/N . The standard error of difference between the means was taken as $\sqrt{\frac{\sigma_I^2}{n_I} + \frac{\sigma_{II}^2}{n_{II}}}$ where the subscript numerals refer to the respective groups of dogs.

RESULTS. I. *Effects on Renal Function.* a. *Maintenance of function.* That renal function is not impaired by the anesthesia was suggested by values of diodrast and inulin clearance which in 34 of 37 experiments were within the ranges we consider normal for unanesthetized dogs. Proof of this maintenance of function is shown in table 1, in which are summarized observations in 4 dogs in which observations were available during the conscious state. The only deviations observed during anesthesia from values obtained while conscious are (1) in 4 of 8 instances lower rates of urine flow, and (2) in all 8 observations, an increase of arterial pressure.

b. *Occasional failure of renal function.* Evidence of temporary failure of

TABLE 1

Effect of anesthesia on renal function

Absence of effect on pentobarbital anesthesia on renal function. Each observation of clearance or Tm_D recorded is the mean of three periods of urine collection each of about 10 minutes' duration.

DOG NO.	DATE	ANESTHETIZED OR CONSCIOUS	CLEARANCE		FILTRATION FRACTION	URINE VOL.	HEMATOCRIT	ARTERIAL PRESSURE	Tm
			Diodrast	Inulin					
1	8-14	Anes.	cc. per min. 172	cc. per min. 70	0.41	cc. per min. 0.5	per cent 44	mm. Hg 182	14.4
	8-17	Conse.	165	69	0.42	0.5	39	144	
	12-21	Anes.	170	53	0.31	0.5	50	198	
	12-30	Anes.	165	54	0.33	0.2	40	160	
2	8-21	Conse.	135	46.4	0.34	0.3	38	106	9.6
	8-24	Anes.	111	43.6	0.39	0.5	41	170	9.9
	8-27	Conse.	107	35.5	0.33	0.6	36	120	9.7
	9-15	Anes.	103	35.6	0.33	0.1	36	155	
	10-6	Conse.	119	39	0.33	0.3	39	101	
3	6-30	Conse.	286	78	0.27	0.4	42	128	16.5
	7-6	Conse.	238	76	0.32	0.6	44	129	16.9
	12-6	Anes.	248	81	0.32	0.2	56	158	17.2
4	6-18	Conse.	259	75	0.29	0.5	48	128	16.4
	7-1	Conse.	173	61	0.35	0.8	50	142	17.5
	5-31	Anes.	189	67	0.36	0.3	43	150	16.9
	6-7	Anes.	221	62	0.28	0.5	41	152	18.9

TABLE 2

Depression of renal function during anesthesia

Observations in 2 dogs showing failure of renal function under pentobarbital anesthesia; in one case (a) observations under anesthesia are averaged from 3 periods of urine collection and compared with similar averages from the conscious state; in the other (b) progressive failure of function is shown in succeeding 10 minute periods.

(a) Dog 5

DATE	ANESTHETIZED OR CONSCIOUS	CLEARANCE		FILTRATION FRACTION	URINE VOLUME	HEMATOCRIT	ARTERIAL PRESSURE
		Diodrast	Inulin				
		cc. per min.	cc. per min.		cc. per min.	per cent	mm. Hg
2-26	Conscious	210	61.8	0.29	0.7	44	134
3-8	Conscious	231	64.3	0.28	0.5	42	128
4-27	Anesthetized	47	not done		0.1	51	176

(b) Dog 6

PERIOD OF URINE COLLECTION	DURATION OF COLLECTION						
	minutes						
1	10.5	308	72.5	0.23	0.2	42	124
2	10.0	210	48.8	0.24	0.15		118
3	10.75	98	26.8	0.27	0.1		116

renal function was obtained in 3 dogs of 37 on which observations were made under anesthesia. In one of these (no. 5) observations had been made during the conscious state and the contrast with the effect of anesthesia is shown in table 2a; in another (no. 6) the onset of renal failure under anesthesia is shown in succeeding periods of urine collection (table 2b).

II. *Effect of Arterial Pressure.* In the nine experiments shown in table 1 and table 2a the arterial pressure is consistently greater under anesthesia. This characteristic of pentobarbital anesthesia is not generally recognized. For this reason the data from a large series of experiments were submitted to the analysis described above and summarized in table 3.

TABLE 3

Summary of observations of blood pressure and calculations from (group I) conscious trained dogs and (group II) normal dogs under pentobarbital anesthesia

	GROUP I CONSCIOUS DOGS	GROUP II ANESTHETIZED DOGS
Number of observations.....	585	150
Number of animals.....	27	145
Mean level of arterial pressure, mm. Hg.....	129	146.7
Standard deviation, mm. Hg.....	± 4.8	± 21.1
Standard deviation of mean, mm. Hg.....	± 0.9	± 1.7
Standard error of difference.....	0.99	
Difference of means	17.95	
Standard error of difference.....		

DISCUSSION. Our observations show that renal function is usually maintained at normal levels during pentobarbital anesthesia and that blood pressure is usually increased. But it must be realized that these conclusions apply only to conditions of dosage and mode of administration similar to those used in these experiments. Indeed, the occasional failure of renal function in this group suggests that even with the procedure in use we are still on the threshold of toxicity.

The maintenance during anesthesia of normal values of plasma diodrast and inulin clearances and of maximum capacity to excrete diodrast by secretion indicate respectively that renal plasma flow, glomerular filtration rate and one of the tubular functions of the kidneys are unaltered (Smith, 1937; Smith, Goldring and Chasis, 1938). We have shown that the proportion of diodrast extracted from plasma by the kidney is not depressed during anesthesia (Corcoran and Page, 1943; Corcoran, Taylor and Page, 1943) and it follows from this and the maintenance of clearance that the rate of renal blood flow is not altered. But it cannot be concluded that renal circulation is entirely unchanged, for the absence of change of renal blood flow during the increased blood pressure indicates that renal resistance must have been increased. Filtration fraction, i.e., the ratio of inulin to diodrast clearance, is not affected by anesthesia, a fact which suggests that the increased renal resistance is not

associated with a change in the head of pressure within the glomerular capillaries, for a change in filtration pressure would alter the volume of filtrate formed per unit volume of plasma flow through the glomeruli. The site of increased resistance is therefore probably the afferent arterioles, constriction of which would at once maintain renal blood flow and intraglomerular pressure at normal levels during an increase of arterial pressure. This activity of the afferent arterioles is probably an expression of their participation in the general tendency of the renal circulation to maintain itself unchanged during variations of arterial pressure (reviewed by Smith, 1939). That the vasoconstriction is more likely the result than the cause of the increase of arterial pressure is suggested by its locus in the afferent arterioles, for most types of peripheral vasoconstriction which increase arterial pressure are associated with an increase of filtration fraction and evidence of efferent arteriolar constriction, e.g., psychogenic constriction, adrenin (Smith, 1939) renin, angiotonin (Corcoran and Page, 1939, 1940). The homeostasis of renal circulation during the increase of arterial pressure caused by pentobarbital anesthesia thus resembles the renal state during the cardiac hypertension released by injection of atropine and infusion of pitressin (Corcoran and Page, 1939).

The mechanism of the renal failure which occasionally develops under anesthesia is not clear. Our observations suggest that renal plasma flow and glomerular filtration rate are concurrently depressed, while the oliguria, sometimes present when these functions are not altered, is increased. The failure is not the result of arterial hypotension, for it occurred in one animal (no. 5) whose arterial pressure was increased by anesthesia, while in another clearance fell by two-thirds during an 8 mm. Hg decrease of arterial pressure. Since renal plasma flow is decreased, the cause may lie in renal vasoconstriction similar to that observed by Haury, Gruber and Gruber (1939) during intravenous injection of thiobarbiturate. This explanation must remain speculative until simultaneous observations of total renal blood flow and diodrast clearance have confirmed it. Alternatives suggest themselves such as a breakdown of tubular barriers due to concentration of the anesthetic in tubular fluid.

The increase of arterial pressure observed in the 9 experiments of tables 1 and 2 is confirmed as a general reaction to pentobarbital anesthesia by the data of table 3. The differences of mean level of pressure between the conscious and anesthetized dogs may be considered statistically convincing, since this value divided by the standard error of the differences greatly exceeds the value of 3 usually accepted as the test of significance. The increase in pressure may be attributed to sympathetic excitation similar to that which occurs in other types of anesthesia (reviewed by Smith, 1939). Whatever its cause, the fact that it occurs should revise upwards the "normal" levels of arterial pressure to be expected in dogs given pentobarbital sodium as an anesthetic. The level of pressure is a widely accepted indicator of the general condition of anesthetized animals and it is therefore important to realize that, under pentobarbital, levels of 110 or even 120 mm. Hg measured by mercury manometer may be indications of toxic depression. Since renal failure may occur without a decrease of

arterial pressure, it may be that observation of urine flow will serve as an additional and possibly more delicate safeguard in experiments which the depressant effects of anesthesia might disturb.

SUMMARY

Anesthesia induced in dogs by intraperitoneal administration of 30 mgm. per kilo body weight of pentobarbital sodium does not usually impair renal function, for diodrast clearance and tubular secretory capacity as well as inulin clearance may be unaltered as compared with values observed in the resting conscious state. The maintenance unaltered of effective renal plasma flow (plasma diodrast clearance) and filtration fraction during the increase of arterial pressure induced by anesthesia indicates that the afferent arterioles have moderately constricted, in response, not to the anesthesia, but to the hypertension it causes. When renal failure occurs, it is associated with marked oliguria and with concurrent depression of diodrast and inulin clearances independently of changes in arterial pressure. Attention is drawn to the increase of arterial pressure commonly present during pentobarbital anesthesia in dogs. Levels of 110 to 120 mm. Hg which might be accepted as normal in conscious dogs may express toxic depression under pentobarbital anesthesia. It is suggested that the onset of severe oliguria, indicating as it does the onset of renal failure, may provide a more delicate index of the toxic effects of the anesthetic than does a decrease of arterial pressure.

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THE EFFECT OF VITAMIN A ON SOME RENAL FUNCTIONS OF THE DOG

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A series of papers have been published in recent years indicating that the feeding of large doses of vitamin A increases renal function in the dog (1) and man (2). Furthermore, reports describing a fall in blood pressure after administration of vitamin A in hypertensive subjects (3) indicate that a possible correlation might exist between the renal action of this substance and the mechanism responsible for its hypotensive effect. The experiments reported in this paper, carried out over a period of two years, were designed to investigate the effect of various doses of vitamin A on the filtration rate, effective renal plasma flow and tubular excretory mass of normal dogs, maintained on a basal diet.

METHODS. All experiments were performed on a total of seven unanesthetized female dogs. The animals were maintained during the whole period of observation on a daily diet consisting of 100 grams crackermeal, 30 grams meat powder,² 30 grams skimmed milk, 3 grams salt mixture and 5000 units of vitamin A.³ Diodrast clearances (4) were used in six, and p-aminohippuric acid clearances (5) in five experiments to measure the effective renal plasma flow. The maximal tubular capacity for the excretion of diodrast (6) was determined in six dogs, p-aminohippuric acid being used in the rest of the animals. Before feeding large doses of vitamin A, normal control values for creatinine, diodrast or p-aminohippuric acid clearances and diodrast or p-aminohippuric acid Tm were established by performing weekly clearances over a period ranging from two to three weeks. Immediately following this control period the feeding of large doses of vitamin A was begun. Fifty thousand units were given to 3 animals, the other 4 receiving 200,000 units, daily. The large doses were continued over a period ranging from two to three months; renal function as described above was determined at bi- or tri-weekly intervals. Following this experimental period, the dosage was reduced to the initial control level of 5,000 units, and the clearance values were again observed for an additional period of three months.

EXPERIMENTAL. The effect of the oral administration of 50,000 units of vitamin A on the renal clearances was inconsistent (table 1). In one animal a slight rise in diodrast Tm values above the control was observed. However, since the diodrast Tm fell while the high dosage was being maintained, the rise was perhaps not caused by the vitamin. In another dog diodrast Tm values remained at the control levels, and in a third animal they rose slightly from 12.6

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² Valentine Meat Company.

³ E. R. Squibb and Sons.

to 15.8 mgm. diodrast iodine per minute. Since in this last case a further rise was observed 26 days after the high dosage was discontinued, it is probable that

TABLE 1
Effect of 50,000 units of vitamin A

NO. OF DOG	SURFACE AREA	VITAMIN A PER DAY	DAY OF EXPERIMENT	PLASMA CLEARANCES		T _m DIODRAST	FILTRATION FRACTION
				Creatinine	Diodrast		
	sq. m.	units		cc./min.	cc./min.	mgm. iodine per min.	
3	0.691	5,000	5-22-42	78.8	271.0	21.1	0.290
			7-24-42	79.9	206.7	20.5	0.385
		50,000	8-14-42	63.6	194.5	22.17	0.326
			9- 5-42	70.2	214.8	20.2	0.328
		5,000	9-19-42	74.3	209.8	18.95	0.354
			10-10-42	76.3	268.8	19.20	0.284
5	0.775	5,000	4-17-42	82.0	242.0		0.290
			4-20-42			12.1	
			5-24-42	44.6	162.0	15.8	0.295
		50,000	6- 9-42	73.5	205.0	15.8	0.295
			8- 3-42	89.1	214.8	14.5	0.350
			8-12-42	54.6	160.0	14.7	0.341
		5,000	9- 8-42	71.4	213.0	17.5	0.335
			9-15-42	70.8	140.0	15.8	0.372
			9-30-42	79.4	220.0	19.0	0.360
			10-27-42	77.1	274.0	18.7	0.281
			12-29-42	79.9	306.0		0.260
6	0.450	5,000	1-23-42	37.6	114.0		0.329
			1-28-42	34.8	111.0	9.5	0.314
			2- 4-42	36.8		7.8	
		50,000	2-12-42	38.5	145.0	9.6	0.266
			2-20-42	42.4	145.0	11.6	0.292
			2-26-42	50.0	148.0		0.338
			3- 4-42	44.6	158.0	10.1	0.282
			3-18-42	32.3	161.0		0.201
			3-20-42	43.4	123.0	13.4	0.353
			3-25-42	56.0	175.0		0.320
			3-27-42	32.5		7.70	
		5,000	4- 8-42	43.4	152.0		0.285
			6-16-42	49.0			
			6-18-42	51.6	132.0	9.7	0.391

the increase in the T_m value was either the result of normal variation or the response to threshold dosages. The data obtained on the filtration rate and

TABLE 2
Effect of 200,000 units of vitamin A

NO. OF DOG	SURFACE AREA	VITAMIN A PER DAY	DATE OF EXPERIMENT	PLASMA CLEARANCES			Tm		FILTRA-TION FRACTION
				Creatine	PAH	Diodrast	PAH	Diodrast	
	sq. in.	units		cc./min.	cc./min.	cc./min.	cc./min.	mgm. iodine per min.	
1	0.683	5,000	11-24-42	62.5	184.0		16.5		0.340
			11-30-42	46.1	141.8		16.2		0.325
		200,000	12-21-42	61.0	157.5		17.9		0.387
			2-11-43				19.0		
			3-17-43	66.0	188.2		28.3		0.350
		5,000	4- 5-43	72.4	216.0		19.5		0.336
			5-12-43	77.4	226.0				0.342
			5-21-43	44.3	116.8				0.379
			5-24-43	66.5	208.0				0.319
2	0.715	5,000	11-24-42	52.2	223.5		10.4		0.260
			12- 1-42	59.8	175.7		12.0		0.340
		200,000	1- 6-43	47.6	158.9		17.9		0.299
			3-22-43	68.3	247.5		20.5		0.276
		5,000	4-24-43	47.7	239.9		20.6		0.199
			5-10-43	49.6	164.0		20.2		0.210
			5-26-43	55.3	257.0		16.2		0.216
			6- 2-43	39.8			14.7		
3	0.691	5,000	9- 5-42	70.2	214.8		20.2		0.327
			9-19-42	74.3	209.8		19.0		0.354
		200,000	10-13-42	76.9	219.0		24.6		0.351
			11- 9-42	83.9	279.0		29.7		0.306
			12- 3-42	85.0	283		28.5		0.300
		5,000	1- 8-43	92.8	297.0		29.5		0.312
			1-30-43	76.6	226.0		20.4		0.339
4	0.765	5,000	9-11-42	66.5		214.0		18.9	0.311
			9-22-42	66.1		199.0		18.1	0.321
		200,000	10-16-42	77.0		212.0		21.9	0.363
			11-17-42	75.5		233.0		25.6	0.324
		5,000	12-15-42	62.7		221.0		24.1	0.284
			1-11-43	51.2		174.3		22.1	0.293

the effective renal plasma flow were equally inconsistent showing either no change or fall during the experimental period (table 1). In one instance the creatinine and the p-aminohippuric acid clearances rose following the discon-

tinuation of the high vitamin A dosage (table 1). It was evident, therefore, that the feeding of 50,000 units of vitamin A had no significant effect upon the renal functions observed. This conclusion was further confirmed by statistical treatment of these data. Table 3 shows the maximal range for the normal variation obtained statistically from 32 observations, each consisting of two collection periods. Twelve of these observations were obtained with p-aminohippuric acid, the rest with diodrast. The highest values obtained for each individual animal during the ingestion of 50,000 units lie below the maximal range.

The effect of the daily addition of 200,000 units of vitamin A to the standard diet of 4 dogs resulted in a significant rise in the maximal tubular capacity for the excretion of diodrast or p-aminohippuric acid, the increase averaging from 50 to 100 per cent of the control value (table 2). Figure 1 representing a typical experiment shows that the administration of 200,000 units was effective within

TABLE 3
Deviation of experimental T_m data from maximal normal range

NO. OF DOG	NO. OF UNITS VITAMIN A GIVEN	SURFACE AREA	AVERAGE OF HIGHEST EXPERIMENTAL POINTS		MAXIMUM RANGE FROM CONTROL DATA	
			T_{mD}	T_{mPAH}	Diodrast	PAH
		<i>sq. in.</i>				
1	200,000	0.683		41.4		28.4
2		0.715		28.7		28.4
3		0.691		43.0		28.4
4		0.765	33.4		35.2	
5	50,000	0.775	20.4		35.2	
3		0.691	32.7		35.2	
6		0.450	29.8		35.2	

a period of three weeks. Similar results were obtained in 2 other dogs. In the fourth animal the rise in T_m did not appear until after five weeks. When these data are treated statistically in the manner described above it is seen that the highest T_m figures per square meter of body surface, as obtained on three dogs, exceeded the maximal range of the control data, indicating a significant increase in the renal excretory mass (table 3). In the fourth animal the highest T_m figure lay within the normal variation. Upon individual treatment of the data obtained on this animal, however, it can be seen that a rise in the T_m value above its control data resulted from the application of the vitamin (table 2). A rise was observed in the filtration rate and the effective renal plasma flow of all but one dog, in which the high vitamin A dosage produced no effect. Since this increase ranged from 5 to 30 per cent of the control figures, it was less than the increase observed for the corresponding T_m values. It seems of special significance that the filtration fraction did not change during the vitamin A hyperemia, an observation confirming the results of Corcoran and Page (2). The effect

of vitamin A differs, therefore, from that produced by pyrogens (7) or yeast adenylic acid (8) in which the filtration fraction falls during the hyperemic period.

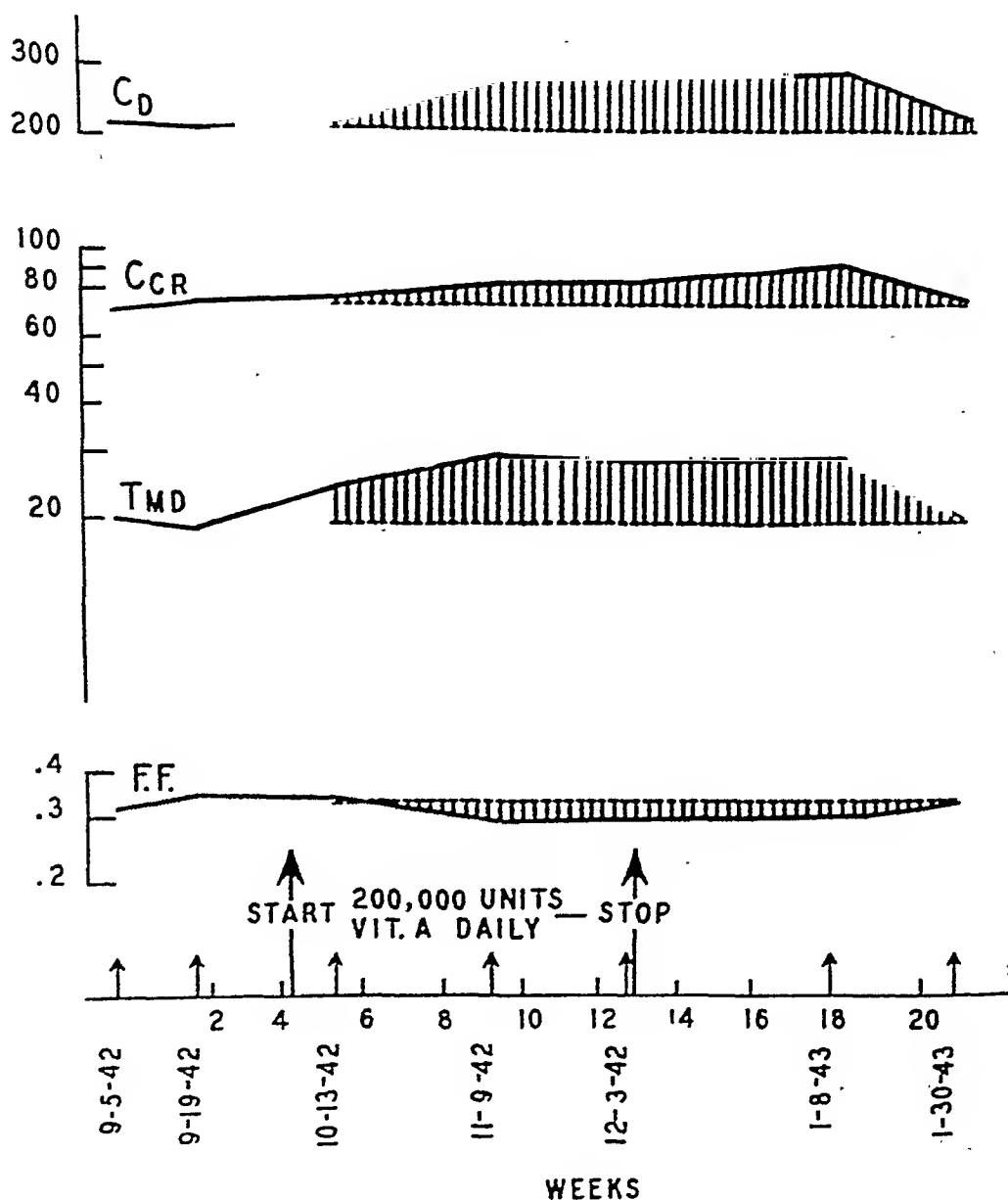


Fig. 1 demonstrates the effect of the daily administration of 200,000 units of vitamin A on the effective renal plasma flow, C_D , the filtration rate, C_{CR} and the maximal tubular excretory capacity for diodrast, T_{MD} .

DISCUSSION. The experiments reported in this paper indicate that the addition of 5,000 as well as 50,000 units of vitamin A has no significant effect upon the glomerular filtration rate, effective renal plasma flow or tubular excretory mass, while the daily administration of 200,000 units causes a significant increase in T_m values in all animals observed. The renal plasma flow and the filtration rate showed a moderate increase in three animals and no change in the fourth.

The rise observed after these large doses is not attributable to correction of avitaminosis, since the dogs had been maintained on a presumptively adequate vitamin intake during the control period. It is also improbable that the rise was caused by free essential fatty acids, since the corn oil used as vehicle for the vitamin contained no fatty acid radicals (9).

From tables 1 and 2 it will be seen that the filtration fraction remained constant throughout the period of hyperemia. This fact indicates that the filtration pressure in the glomerulus remains constant if it is assumed that filtration equilibrium is reached in the glomerular capillaries (10). It is probable, therefore, that the changes in renal blood flow produced by the vitamin A must be mediated by changes in both the afferent and efferent glomerular arterioles. Consequently the mechanism of renal hyperemia described in this paper is similar to that observed to follow the feeding of fish to the harbor seal (11) and of a high protein diet to normal and hypertensive dogs (12).

The effect of large doses of vitamin on the maximal rate of diodrast and p-aminohippuric acid excretion resembles the action of testosterone on the kidney of dog and man (13). In contrast to testosterone, however, vitamin A caused an increase in the effective renal plasma flow and filtration rate in three out of four animals. The rise in the T_m figures may indicate the development of a true renal hypertrophy, an assumption supported by the work of Korenchevsky and others (14) who found that testosterone produces a significant increase in kidney weight. It is possible that the rise in plasma flow and filtration rate observed after the feeding of 200,000 units of vitamin A represents a circulatory adjustment of the kidney to the increased tubular mass of that organ.

SUMMARY

The oral administration of 5,000 and 50,000 units of vitamin A produces no significant change in the glomerular filtration rate, the effective renal plasma flow and the tubular excretory capacity for diodrast or p-aminohippuric acid of normal unanesthetized dogs kept on a standard diet.

The feeding of 200,000 units of the vitamin results in a significant rise in the tubular excretory mass, and a moderate increase in renal plasma flow and filtration rate. No change in filtration fraction occurs during the hyperemia.

The relation of vitamin A to other renotropic substances is discussed.

I wish to express my gratitude to Miss Christine Waples and Mrs. Louise Buchanan for their assistance in performing the experiments.

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THE EFFECT OF ATROPINE AND QUINIDINE SULPHATE ON ATROPHY AND FIBRILLATION IN DENERVATED SKELETAL MUSCLE¹

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Fibrillation was first observed by Schiff (10). Langley and Kato (7) ascribed the loss in weight following motor denervation to this activity. Having inhibited fibrillation effectively with quinidine sulphate, and finding little change in atrophy, Solandt and Magladery (11) questioned the overwork hypothesis of Langley (6). Soskin and co-workers (9, 12), using atropine sulphate to decrease fibrillation and physostigmine to increase fibrillation, obtained weight changes in denervated muscle which they interpreted as supporting Langley's hypothesis. The present work was undertaken to obtain new evidence on the relation of atrophy to fibrillation in denervated muscle.

METHODS. One hundred and fifty male albino rats (average weight 220 grams) were used. Denervations were carried out by sciatic nerve section high in the thigh. The choice of the leg to be denervated and the assignment of animals to the treatment to be received were made at random, using Tippett's Random Sampling Numbers (13). Five groups of animals were designated as follows:

- (1) Thirty-seven rats to receive atropine sulphate 38 mgm. daily per 100 grams.
- (2) Thirty-seven rats to receive quinidine sulphate 38 mgm. daily per 100 grams.
- (3) Twenty-six rats to receive atropine sulphate 19 mgm. daily per 100 grams.
- (4) Twenty-six rats to receive quinidine sulphate 19 mgm. daily per 100 grams.
- (5) Twenty-four rats to receive saline.

A larger number of animals was assigned to be given large doses (38 mgm./100 grams) of atropine and quinidine sulphate because of the higher mortality to be expected in these categories. Moderate doses (19 mgm./100 grams) of the drugs were given to other animals as indicated. In each group the drug was administered subcutaneously and the dose volume in all cases was the same. One half of the daily dose was given at 10 a.m. and the other half at 10 p.m. starting the day after denervation. On the 4th, 8th, 12th, 16th and 20th days after denervation 4 animals were selected at random for test and autopsy from each surviving group.

Fibrillation was recorded using a four-stage valve amplifier and a loudspeaker. Paired electrodes consisting of no. 26 hypodermic needles fixed at a distance of 1 cm. apart were used. One pair of electrodes was inserted into the denervated muscles. The placing of the electrodes was done after careful palpation and observation. A second pair of electrodes was inserted in a like manner into the

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intact muscles of the opposite limb. A double pole double throw switch permitted a ready comparison of the fibrillary potentials of the denervated muscles with the action potentials of normal tonic activity in the intact muscles. In evaluating this difference the output of the amplifier was led to a loudspeaker and the difference in sound intensity between the denervated and the opposite intact groups of muscles was measured using a sound-level meter (General Radio

TABLE 1

DRUG	DAYS AFTER DENER- VATION	FIBRILLATION (ESTIMATED BY SOUND-LEVEL METHOD AND EXPRESSED AS DENER- VATED MINUS INTACT MUSCLE ACTIVITY)	LEG	BODY WEIGHT		MUSCLE WEIGHT	
				Initial	Final	Dener- vated	Intact
				grams	grams	grams	grams
Large quinidine	4	0	Left	262	263	1.38	1.48
	4	2	Left	257	254	1.23	1.51
	4	0.5	Right	232	249	1.42	1.55
	4	0	Left	220	200	1.18	1.25
Large atropine	4	17.5	Right	217	196	0.94	1.16
	4	10	Left	246	218	1.16	1.20
	4	12	Right	256	216	1.26	1.31
	4	14	Right	200	165	0.85	1.01
Moderate quin- idine	4	8.5	Right	198	202	1.19	1.25
	4	7	Left	248	231	1.15	1.21
	4	17.5	Right	180	187	0.86	1.16
	4	16.5	Right	218	230	1.21	1.38
Moderate atro- pine	4	10	Left	264	231	1.22	1.34
	4	12	Right	200	170	0.90	1.00
	4	7	Left	210	189	1.00	1.03
	4	14.5	Right	192	185	1.00	1.14
Saline	4	12	Right	181	193	0.99	1.17
	4	14	Right	266	285	1.51	1.73
	4	17.5	Left	274	266	1.55	1.75
	4	12.5	Left	180	188	0.98	1.15
Large quinidine	8	1	Right	176	158	0.86	0.93
	8	13	Left	193	168	0.72	0.84
	8	6	Right	183	192	0.60	1.08
	8	8	Left	214	206	0.84	1.16
Large atropine	8	5	Right	265	183	0.91	0.91
	8	7	Right	248	190	0.73	0.89
	8	14	Right	238	166	0.52	0.77
	8	13	Right	180	169	0.65	0.97
Moderate quin- idine	8	6.5	Right	186	200	0.87	1.24
	8	9	Left	220	221	1.04	1.42
	8	11	Right	199	230	0.88	1.40
	8	9	Left	240	246	0.96	1.38
Moderate atro- pine	8	4	Right	178	162	0.67	0.87
	8	9	Left	188	181	0.72	1.00
	8	9	Left	250	235	1.08	1.34
	8	10	Right	195	182	0.75	1.04
Saline	8	6.5	Right	194	207	0.97	1.39
	8	14	Left	274	267	1.07	1.76
	8	15	Left	222	237	1.16	1.66
	8	13	Left	274	243	1.04	1.69

TABLE 1.—Continued

DRUG	DAYS AFTER DENER- VATION	FIBRILLATION (ESTIMATED BY SOUND-LEVEL METHOD AND EXPRESSED AS DENER- VATED MINUS INTACT MUSCLE ACTIVITY)	LEG	BODY WEIGHT		MUSCLE WEIGHT	
				Initial	Final	Dener- vated	Intact
				grams	grams	grams	grams
Large quinidine	12	0	Left	188	176	0.56	1.03
	12	2	Left	194	190	0.76	1.08
	12	1.5	Left	202	182	0.57	0.88
Large atropine	12	5	Left	198	165	0.34	0.70
	12	5	Left	175	150	0.43	0.60
	12	15	Right	199	159	0.41	0.66
	12	14	Left	224	163	0.48	0.60
Moderate quin- idine	12	11	Left	233	242	0.41	1.03
	12	3	Right	250	226	0.87	1.30
	12	15	Left	289	300	0.91	1.67
	12	11	Left	255	252	0.87	1.52
Moderate atro- pine	12	16	Left	204	181	0.57	0.97
	12	13	Right	234	181	0.80	1.10
	12	22	Left	211	180	0.69	0.87
	12	15	Left	214	200	0.84	1.22
Saline	12	4	Right	186	243	0.81	1.61
	12	4	Right	286	297	1.01	1.80
	12	7	Right	245	264	0.97	1.68
	12	6	Left	215	228	0.87	1.60
Large atropine	16	2.5	Right	260	187	0.61	0.98
	16	1	Left	228	160	0.49	0.81
Moderate quin- idine	16	0	Left	205	230	0.59	1.30
	16	2.5	Right	180	222	0.66	1.28
	16	2.5	Right	212	193	0.46	1.04
	16	3.5	Right	180	230	0.50	1.31
Moderate atro- pine	16	5	Right	191	178	0.99	0.99
	16	6.5	Right	237	204	1.11	1.11
	16	5	Left	252	172	0.99	0.99
	16	9	Right	229	180	1.21	1.21
Saline	16	10	Right	212	215	0.74	1.70
	16	7	Right	186	235	0.64	1.43
	16	9	Right	219	245	0.75	1.77
	16	2	Right	240	282	0.69	1.75
Moderate quin- idine	20	1	Left	270	254	0.54	1.47
	20	3	Left	260	273	0.62	1.56
	20	2	Right	205	212	0.49	1.13
	20	11	Left	255	254	0.49	1.61
Moderate atro- pine	20	12	Right	241	215	0.40	1.07
	20	5	Right	211	199	0.57	1.11
	20	8	Left	265	187	0.53	0.99
Saline	20	6	Left	180	274	0.59	1.62
	20	3	Left	210	247	0.67	1.68
	20	8	Right	221	260	0.80	1.79
	20	3	Right	256	288	0.59	1.98

—Type 759A). The sound-level meter served the purpose of an output meter, sufficiently damped to give an integrated reading of the amplifier output. The

use of a suitably damped output meter would have served the same purpose and eliminated the frequency response limitations of the loudspeaker which, however, proved unimportant in the present instance. The records were taken under sufficient ether anesthesia to avoid movement on the part of the animal. Controls indicated that the degree of anesthesia, over the range used, did not measurably affect the fibrillation.

The fibrillary potentials were also photographed using a Matthews oscillograph. Fibrillary activity was estimated by counting action potential spikes. This method yielded results quantitatively similar to those obtained by estimating integrated amplifier output with a sound-level meter.

A visual method of estimating the degree of fibrillation was also attempted. Prior to autopsy, fibrillation on the proximal, middle and distal thirds of the anterior and posterior surfaces of the muscle was observed under reflected light.

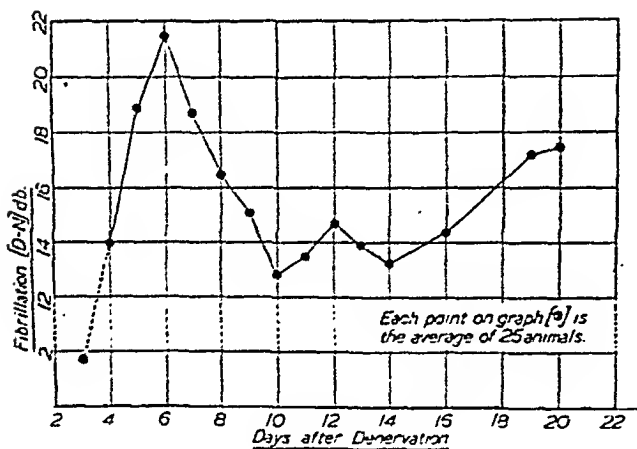


Fig. 1

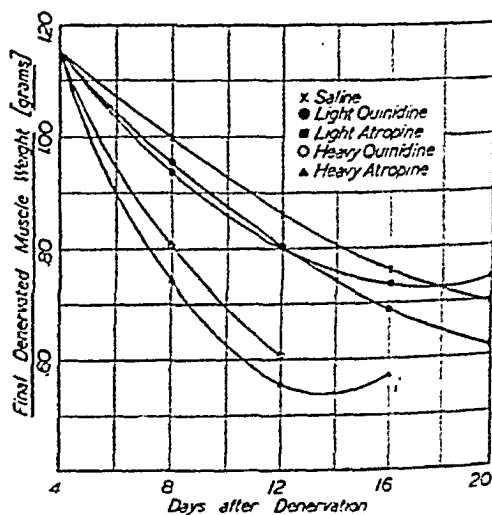


Fig. 2

Fig. 1. Fibrillation in the denervated gastrocnemius-soleus muscle group of the albino rat.

Fig. 2. Denervated muscle weight for each group of rats adjusted to a fixed value of fibrillation and to the same initial body weight.

Activity was recorded as absent (0), slight (1) or marked (2). Visual methods, although lacking accuracy and objectivity, permitted a crude but useful check on conclusions arrived at by the more precise methods described. On limited trials there was a satisfactory correlation between the results obtained using the three methods for estimating fibrillation.

A series of 25 rats, each with one sciatic nerve cut, was studied, using the sound-level method, to follow the fibrillary activity in animals to which no drug had been administered. The average course of fibrillation for this series is shown in figure 1. Fibrillation was readily detected on the 3rd day after denervation. It increased rapidly to a maximum, generally observed on the 6th day, after which it continued prominent for the duration of the experiment (20 days). The initial increase to the maximum reached on the 6th day was remarkably constant. From the 6th until the 9th day a fairly regular decrease was noted. From the 9th to the

20th day the daily records on individual animals showed some variation from day to day.

In the main series of animals, to which drugs or saline were administered, the variables recorded were: (1) Final weight of the denervated muscle (w). (2) Final weight of the intact muscle (i). (3) Initial body weight (y). (4) Final body weight. (5) Amount of fibrillation (x). (6) Duration of the period of denervation (t). (7) Drug, if any, employed (z).

The experiment was intended primarily to investigate the relationship between (1) and (5). The time variable (6) was introduced simply to provide a wider basis for whatever conclusions might be reached regarding this relationship. The various drug applications (7) were used to produce varying degrees of fibrillation. Initial body weight (3) was recorded for the purpose of establishing control over this variable as a potential source of experimental error. The remaining variables

TABLE 2

REGRESSION COEFFICIENTS		t^*
Symbol	Value	
A	0.0771	
B (Time)	-0.1604	-14.5**
C (Time ²)	0.0315	3.6**
D (Fibrillation)	-0.0031	-1.07
E (Initial Body Weight)	0.2882	6.3**
F (Drug)	0.0555	5.55**

* R. A. Fisher, 1934, Section 29.

** Significant at 1 per cent level.

TABLE 3

Final muscle weight		
TREATMENT	REGRESSION COEFFICIENTS	t
Large quinidine	D (Fibrillation)	-0.79
	E (Initial body weight)	0.88
Large atropine	D	-0.73
	E	2.06
Moderate quinidine	D	-0.843
	E	1.596
Moderate atropine	D	0.468
	E	-2.4*
Saline	D	1.359
	E	2.72*

* Significant at 5 per cent level.

(2) and (4) were included to permit an examination of the effect of the drugs when denervation was not present, since any conclusion as to the relationship of (1) and (5) must rest in part on a demonstration that the effects have not been the result solely of the direct action of the drugs.

Two attempts to determine the relationship of atrophy to fibrillation were made. In the first (table 2) a regression equation of form $W = A + Bt + Ct^2 + Dx + Ey + Fz$, where W represents the adjusted value of w , was fitted to show the dependence of w on t , x , y and z (2). A , B , C , D , E and F represent partial regression coefficients. A partial regression coefficient which differs significantly from zero indicates that the corresponding variable exhibits a real relationship with w apart from the effects of the other variables. One which does not differ significantly from zero indicates that the experiment does not give adequate evidence of such a relationship.

A possible objection to this procedure arises from the fact that the variable "drug" is not measurable, and consequently, in order to include it in the analysis,

numbers were assigned arbitrarily to drugs, in such a way that the drug numbers have the same rank as the effect on body weights produced by these drugs. Such a device is not expected to be wholly satisfactory, although it is better than completely neglecting the "drug" factor.

In the second attempt (table 3), this arbitrary element is avoided by fitting a regression equation of form $W = A + Bt + Ct^2 + Dx + Ey$ to the records of each set of rats receiving the same drug. This approach is made possible by the circumstance that fibrillation effects varied considerably within each such set. Differences among these regression equations will presumably indicate differences among the effects of the drugs, but in each of them the fibrillation regression coefficient may be tested as before.

A similar treatment was accorded final intact muscle weight according to the equation $I = A + Bt + Ct^2 + Ey$, where I represents the adjusted value of i , for the purpose of estimating the effect of the drugs when denervation is not present.

In the final evaluation of the effects of body weight (table 4) covariance methods were used (3).

TABLE 4

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES UNADJUSTED	DEGREES OF FREEDOM	SUM OF SQUARES ADJUSTED FOR INITIAL BODY WEIGHT	DEGREES OF FREEDOM	SUM OF SQUARES ADJUSTED FOR INITIAL AND FINAL BODY WEIGHTS
Time.....	2	0.41467				
Drugs.....	4	0.21957**	4	0.16750**	4	0.04138 n.s.
Error.....	8	0.03933	7	0.02877	6	0.02432

** Significant at 1 per cent level.

n.s. Not significant at 5 per cent level.

RESULTS AND DISCUSSION. Table 1 contains the untreated experimental data, obtained in the manner described, and is self-explanatory. Table 2 shows that the regression coefficient associated with fibrillation alone fails to exhibit significance. We have, therefore, no evidence that fibrillation has any effect on the final weight of the denervated muscle. Duration of denervation, initial body weight, and drug effects all prove to be significant factors contributing to the final weight of the denervated muscle. A similar analysis carried out using visual estimations of fibrillation likewise produced a non-significant fibrillation regression coefficient.

In the regression equations fitted to the observations on each drug group, the coefficients denoting fibrillation effects again proved to be non-significant (table 3). Thus these coefficients, although less precise than those of table 2 (since they are based on smaller numbers of animals), lead to the same conclusion.

The present experiments thus confirm the original conclusion of Solandt and Magladery (11) that fibrillation is not the cause of the atrophy observed in denervated skeletal muscles. Lazere, Thomson and Hines (8) and Weddell, Feinstein and Pattle (14) have presented evidence which supports this conclusion.

The equations for each drug group also demonstrate, through the differences

between them, that the responses to the various drugs were substantially different. The curves of W against t , for fixed values of x and y , are shown in figure 2. These curves indicate the effect of the treatments used on the weight of the denervated muscle. The least final denervated muscle weight was obtained with large doses of atropine. Large doses of quinidine did not depress the final weight of the denervated muscle quite so far and moderate doses of atropine, quinidine and saline were progressively better in that order. The upward trend in final muscle weight shown by the rats receiving large doses of atropine on the 16th day and those receiving moderate doses of atropine on the 20th day is probably due, in

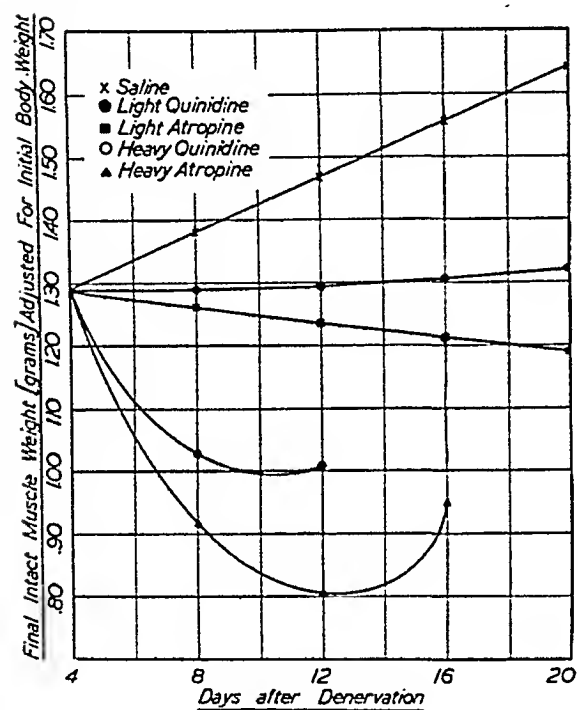


Fig. 3

Fig. 3. Final intact muscle weight for each group of rats adjusted to the same initial body weight.

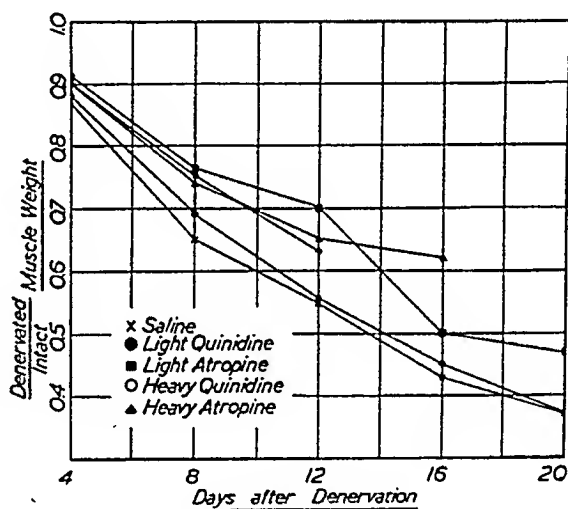


Fig. 4

Fig. 4. Showing the inversion of true results obtained by plotting the ratio denervated over intact final muscle weight in an experiment involving the use of procedures affecting both muscles.

part at least, to the circumstance that unusually heavy rats contributed these points on the graph. The first degree adjustment performed in these cases might not be entirely adequate. Even if this late weight recovery is a real effect, possibly due to accommodation to the drug, it is one shared by both intact and denervated muscles. These experiments give no support to the view that atropine sulphate has a selective beneficial effect on denervated muscle.

Characteristic changes in body weight were seen with each treatment used. Rats receiving saline demonstrated an increase, as was to be expected in growing animals, and this increase was also in evidence when moderate doses of quinidine were administered. Large doses of quinidine and moderate doses of atropine

resulted in some loss while large doses of atropine resulted in a profound loss of body weight. Therefore the question may be raised as to whether, for example, the severe atrophy of denervated muscle exhibited by rats receiving large doses of atropine was the result of action by the drug on the denervated muscle, or if this weight loss simply reflected a general loss of body weight. Some light was thrown on this question by the curves of final intact muscle weight plotted against time, adjusted for varying initial body weight according to the equation $I = A + Bt + Ct^2 + Ey$. These curves (fig. 3) demonstrate that at least a large part of the weight loss of the denervated muscle is also exhibited by the intact muscle. Consequently much if not all of the difference, between one drug and another, in weight loss of the denervated muscle must be attributed to difference in loss of body weight produced by those drugs.

Levine, Goodfriend and Soskin (9) and Soskin and Levine (12) have reported phenomenal weight retention in the denervated muscles of animals receiving large doses of atropine sulphate. In these experiments the ratio of the weight of the denervated muscle to the weight of the corresponding intact muscle from the opposite limb is used as a measure of the existing atrophy. The data of our present experiments, using this ratio, are plotted in figure 4. Here it would appear that moderate doses of quinidine and atropine and large doses of these same drugs, in this order, are progressively more effective than saline in retarding weight loss in the denervated muscle. However, figures 2 and 3 show that animals yielding high values of this ratio are exhibiting high weight loss of the intact muscle, not low weight loss of the denervated muscle. Fischer (1) corroborates this point. Hines, Thomson and Lazere (4) have pointed out that the use of the intact member as a control in drug studies is inadvisable. Knowlton and Hines (5) likewise showed that in cases where the animals undergo significant changes in body weight after denervation the assumption that the control muscle represents the initial weight of the denervated muscle is untenable. Solandt and Magladery (11) used this ratio to indicate the extent of atrophy. The method did not lead to erroneous conclusions in this case because the dose of the drug used was not large and the body weight loss slight as compared to the loss exhibited in the present experiments.

Loss of body weight may account for the whole of the differences between drug effects. This possibility may be tested by adjusting these differences for variations in both initial and final body weight using covariance methods. In order to avoid gaps in the data, observations made after the 12th day were deleted. Table 4 shows the sums of squares of deviations from the mean, of the average weights of denervated muscle for the 5 treatments used, unadjusted, adjusted for initial body weight, and adjusted for both initial and final body weights. When the differences in initial body weight are accounted for the treatment differences are still significant, but when both initial and final body weights are taken into account the treatment differences are no longer significant. We conclude that whatever differences have been observed among the weights of the denervated muscles under different drug applications can be accounted for on the basis of loss in body weight. It appears, therefore, that the drugs have had no demonstrable specific effect on the weight of the denervated muscles.

SUMMARY

1. The atrophy of denervated skeletal muscle is not produced by fibrillation nor is it significantly affected by fibrillary activity.

2. Large, repeated doses of atropine or quinidine sulphate give rise to a reduction in animal body weight and this includes weight-loss on the part of both intact and denervated skeletal muscles.

3. Neither atropine nor quinidine sulphate exerts any specific effect on the weight of denervated skeletal muscle.

The authors appreciate the interest which Prof. C. H. Best has taken in this work.

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THE EFFECT OF TEMPERATURE OF THE BLOOD ON THE HEART RATE¹

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The effect of variations in the temperature of the blood flowing through the heart in the heart-lung preparation was studied in the dog by Knowlton and Starling in 1912 (1). Under the conditions of that experiment, the temperature of the venous blood of the heart was the only factor affecting the pulse rate; the rate was unaffected by changes in the arterial or venous pressure. When the temperature of the blood lay between 26.5°C. and 43.0°C., the pulse varied from 63 to 165, and bore an approximately linear relation to the temperature. The heart rate decreased when the temperature of the blood exceeded 43.0°C. Kisch, in 1923 (2), by means of an arterio-venous anastomosis through a paraffin-lined cannula kept in a water-bath between 50°C. and 60°C., noted a rise of 20 to 40 beats in the pulse of rabbits for each 0.5°C. rise in the temperature recorded in the mediastinum. Similar experiments in dogs showed a ten-beat increment per 0.5°C. rise in the temperature recorded in the right auricle. The effect of cooling the blood was not investigated. Section of the vagus nerves or the sympathetics did not abolish the tachycardia produced under these experimental conditions.

In the course of studies unrelated to the problem discussed here there were observed alterations in the heart rate which appeared to be associated with variations in the temperature of even small amounts of fluid injected into the external jugular vein. The paucity of data concerning the effect of temperature of the blood on the heart rate of the intact animal led us to investigate this problem further. Records were made, therefore, of the alterations in the heart rate produced by injection into the external jugular vein of the intact animal of fluid, the temperature of which was varied from 22.0°C. to 57.0°C.

METHODS. Five rabbits and six cats, all healthy adult animals weighing between 3 and 3.5 kgm., were used. Pulse and blood pressure were measured by means of a glass cannula in the left carotid artery connected to a Huerthle membrane manometer and recorded kymographically. Venous pressure was measured with the aid of a cannula in the right external jugular vein, pointing cephalad, and recording in similar fashion. Another cannula was placed lower in the same vein, pointing toward the heart, for the purpose of infusion. Both membrane manometers were calibrated in millimeters mercury. Respirations were recorded from the side arm of the tracheal cannula by means of a Marey tambour. Preparation for the experiment included intravenous injections of heparin, 150 units per kilogram. Three rabbits were anesthetized with sodium pentobarbital (30–35 mgm. per kgm. given intraperitoneally) and two with

¹ This investigation has been aided by a grant from the Josiah Macy Jr. Foundation.

novocaine infiltration of the skin and subcutaneous tissues of the neck. Three cats received sodium pentobarbital in the same dosage and by the same route; three were given light intratracheal ether anesthesia with no premedication.

Physiologic solution of sodium chloride was employed in all experiments. Infusions were given from a 250 cc. infusion flask, suspended approximately

TABLE 1

Pulse variations from the normal caused by infusions of normal saline at different temperatures

	TEMPER- ATURE OF SALINE INFUSED	ANIMAL—RABBIT	ANIMAL—CAT
Total 69	°C. 22-26	-60 -80	-40 -50 -40
	27-31	-30 -40 -40 -50 -50 -60 -60 -60 -60 -60 -100 -100	-10 -10 -10 -20 -20 -20 -20 -20 -20 -20 -30 -30 -30 -30 -30 -40 -40 -40 -40 -40 -50 -60 -60
	32-36	-30 -30 -30 -30 -40 -40 -40 -40 -50 -50 -50 -60 -60 -60	-10 -10 -10 -10 -20 -20 -20 -20 -20 -20 -30 -30 -30 -30
Total 39	37-40	-10 -10 000 000 000 000 000 +5 +5	-5 -10 -10 000 000 000 000 +10 +10 +10 +10 +10
Total 31	41-45	+10 +10 +10 +20 +20 +20 +30	000 +5 +10 +10 +20 +20 +20
	46-50	+20 +40 +50	+10 +20 +20 +40
	50-51	+40 +50 +50 +60	+20 +20 +30 +40

one meter above the level of the cannula and connected to it by means of transparent rubber tubing. A thermometer was held in place in the flask at the point of outflow of the fluid. The tubing connecting the flask and the cannula held about 15 cc. of fluid. Because of the cooling which occurred in this rubber tube (from 40°C. to 33°C. in 1-2 min.) it was necessary to empty it immediately

prior to each infusion. This was accomplished by interposing a three-way stopcock in the system just above the cannula, and allowing 30 cc. of fluid to escape from the sidearm.

Since the fluid beyond the stopcock could not be drained, 2 cc. of saline solution were allowed to run slowly into the jugular vein a few seconds before every test infusion, to exclude the effect of fluid of unknown temperature. The infusions were given at rates which varied from 150 to 200 cc. per minute and from 2 to 25 cc. in amount. Precautions were taken to prevent overloading of the vascular system in the course of the experiments. A total of 69 cool (22°C. to 36°C.) and 31 warm (43°C. to 57°C.) infusions were given. These were controlled by 39 infusions of fluid at "body" temperature (within 2.0°C. above or below the rectal temperature). In almost all experiments observations were made both before and after vagotomy.

The results of these experiments may be listed:

1. Infusions of saline solution at "body" temperature produced no change in the heart rate.
2. Infusions of saline solution at a temperature below that of the body produced a bradycardia in all experiments. This, depending upon the temperature of the infusion, varied from 20 to 100 beats.
3. Infusions of saline solution at a temperature above that of the body caused a tachycardia of from 10 to 90 beats.
4. These effects occurred equally well both before and after bilateral cervical vagotomy.
5. A number of infusions of cool saline (30°C. – 35°C.) given into the femoral vein and into the pulmonary artery produced no alteration in the heart rate.
6. In each case the change of pulse rate began 2 to 3 seconds after the beginning of the infusion and lasted for several seconds after all the fluid had been injected. No consistent or important changes in general blood pressure were caused by the infusions. The venous pressure always rose moderately during the administration of the fluid.

DISCUSSION. The generalization may be made that within the limits of the temperatures employed in these studies the lower the temperature of the saline solution the greater the degree of bradycardia, and the higher the temperature the greater the degree of the tachycardia.

The fact that this effect occurs after vagotomy and (as has been shown by Kisch with heat) after sympathectomy indicates probably that local conditions—possibly direct action on the Keith Flack node—are of importance.

The absence of alterations in the pulse rate when saline solution was introduced into the femoral vein and pulmonary artery suggests that in these instances mixing and warming of the fluid injected take place in its transit to the right heart.

SUMMARY

In the intact cat and rabbit, the injection of cool physiologic saline solution (22°C. – 36°C.) into the external jugular vein results in a bradycardia and con-

versely the injection of warm saline solution ($43^{\circ}\text{C}.$ - $57^{\circ}\text{C}.$) into the external jugular vein results in a tachycardia. This effect is not altered by bilateral cervical section of the vagus nerves, nor by the action of the several types of anesthetic agents employed.

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THE EFFECT OF THE ADMINISTRATION OF ESTROGEN ON THE MECHANISM OF ASCORBIC ACID EXCRETION IN THE DOG

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It has been demonstrated by Sutton, Kaeser and Hansard (1) that the synthetic estrogenic substance, diethylstilbestrol, causes enhanced urinary excretion of ascorbic acid by rats, animals capable of synthesizing ascorbic acid. The possibility that this might be due to alteration of normal renal tubular function is suggested by the finding of Thorn and Engel (2) that estradiol influences the excretion of sodium and potassium chlorides in dogs. According to these authors, the immediate effect of parenteral administration of estradiol is a decreased excretion of sodium chloride and an increased excretion of potassium chloride, a fact which suggests decreased tubular reabsorption of the latter.

The purpose of the present investigation was to determine if the estrogen, estradiol benzoate, could increase the urinary excretion of ascorbic acid in the dog, which also synthesizes vitamin C. Further, the possibility that such increased urinary excretion might be due to an altered renal mechanism was examined. This could result from a decreased tubular reabsorption of ascorbic acid, a threshold substance cleared by the kidney by glomerular filtration and tubular reabsorption, the latter being limited by a maximal rate (T_m) (3).

It is our experience that the maximal rate of tubular reabsorption (T_m) of ascorbic acid in the dog not only lends itself to fairly precise measurement, but is capable of duplication over a considerable period of time when load levels are adequate to effect tubular saturation (see table 1). In this we are in agreement with Ralli and her associates (3). Accordingly, it is possible to combine daily observations of urinary excretion of ascorbic acid with measurements of ascorbic acid T_m , in order to determine whether increased or decreased excretion is related to changes in tubular function.

METHOD. Four female dogs, ranging in weight from 13.5 to 19.0 kgm., were used. One of the dogs was castrated. Repeated clearances of three to six urine collection periods of ten minutes each were made at various levels of plasma ascorbic acid concentration before, during, and, in two dogs, after injection of estradiol. In all cases the control clearances were made within fifty days before the injection of the hormone. Usually, the desired plasma levels were obtained by constant intravenous infusion, but in some cases subcutaneous injections were used. The simultaneous clearance of creatinine was taken as an index of glomerular filtration rate. Tubular reabsorption of ascorbic acid (mgm./min.) was calculated as the difference between ascorbic acid *load* (glomerular filtration rate \times plasma concentration of ascorbic acid) and its concurrent rate of excretion (urinary concentration \times urine flow in cc./min., or UV).

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These calculations assume that there is no plasma binding of ascorbic acid, which assumption is supported by the fact that at high plasma levels the ascorbic acid

TABLE 1

Ascorbic acid Tm in the dog before, during, and after treatment with estradiol benzoate in a representative experiment (dog RG, wt., 18 kgm.)

DATE	PERIODS	PLASMA ASCORBIC ACID	FILTRATION RATE	LOAD	UV	Tm	$\frac{CAA^*}{CCr}$
Before							
		mgm. per cent	cc./min.	mgm./min.	mgm./min.	mgm. reabs. per min.	
12-19-42	3	2.45	71.0	1.733	1.158	0.575	0.670
12-29-42	4	4.01	67.0	2.692	2.094	0.598	0.776
	4	5.37	78.0	4.200	3.594	0.646	0.825
1-6-43	4	2.85	68.0	1.938	1.528	0.410	0.786
1-8	4	3.93	76.0	3.000	2.500	0.500	0.856
1-9	4	2.50	94.0	2.350	1.838	0.512	0.790
1-12	4	2.37	77.0	1.815	1.330	0.485	0.750
1-14	3	3.40	70.0	2.366	1.738	0.628	0.736
1-16	3	1.58	91.0	1.423	0.913	0.510	0.638
1-21	4	3.95	81.0	3.190	2.535	0.655	0.800
Average.....			77.3	2.471	1.919	0.552	0.763
During							
1-23	4	2.34	100.0	2.340	2.076	0.264	0.880
1-26	3	2.78	103.4	2.880	2.530	0.350	0.880
1-30	3	2.51	74.0	1.860	1.797	0.063	0.974
1-30	4	2.56	85.0	2.155	2.036	0.119	0.944
2-2	4	4.01	86.6	3.465	1.584	0.575†	0.832
2-3	3	2.32	80.0	1.850	2.890	0.266	0.857
2-6	4	1.50	110.4	1.657	1.462	0.195	0.881
Average.....			91.3	2.315	2.054	0.261	0.892
After							
2-10	4	2.03	75.0	1.520	1.220	0.300	0.808
2-13	4	1.93	81.0	1.572	1.201	0.370	0.764
2-17	4	2.72	87.0	2.370	1.910	0.460	0.808
2-20	4	3.33	65.4	2.171	1.808	0.362	0.830
Average.....			77.1	1.908	1.535	0.373	0.802

$$* \frac{CAA}{CCr} = \frac{\text{Ascorbic acid}}{\text{Creatinine}} \text{ clearance ratio.}$$

† During estradiol treatment excessively high load levels gave values for Tm approximating the normal. See discussion.

clearance approaches the filtration rate (3). It was found that a load/Tm ratio of over 2.5 was required to effect complete tubular saturation. Urines were

collected by catheter, and the bladder was washed after low urine flows. The dogs received 30 to 40 cc./kgm. of water prior to the clearance study to insure adequate urine flow.

Plasmas were precipitated for creatinine determination by the CdSO_4 method of Fujita and Iwatake (4), and the creatinine concentration of the plasma filtrate and diluted urines was determined by the method of Folin and Wu (5). Ascorbic acid was determined in both plasma and urine by the dichloroindophenol method of Mindlin and Butler (6)² using a photoelectric colorimeter, with modifications to correct for turbidity as suggested by Bessey (7). In dog F, simultaneous clearances of mannitol and creatinine were made to check on the presumption that creatinine accurately measured the filtration rate during estradiol action. Mannitol determinations were done by the method of Smith, Finkelstein and Smith (8). All analyses were made in duplicate.

Three dogs (two normal and one castrate) were kept in metabolism cages for the determination of the hourly excretion of ascorbic acid before, during, and after treatment with estradiol. To minimize deterioration during 24-hour collections, 5 cc. of concentrated H_2SO_4 were added to the collection vessels, giving a final urine pH of 1.25 to 4.0, depending on the urine volume. Although appreciable deterioration of ascorbic acid occurs when urines are kept under these conditions for long periods at room temperature, nevertheless comparison can be made between control and experimental collections obtained under the same conditions. When ascorbic acid clearances were made during the total urine collection period, the latter was interrupted for an interval of 18 to 30 hours to allow the plasma level of ascorbic acid to return to normal before collections were resumed. Fasting plasma determinations of ascorbic acid were regularly made about 16 hours after feeding. The diet was kept constant in amount during total urine collection periods.³

Since alkalinity influences the rate of destruction of ascorbic acid in urine (9), it was necessary to determine whether estradiol treatment altered urinary pH significantly. A Coleman pH meter was used for this purpose. Regular hematocrit determinations were made on all dogs.

After suitable control periods, estradiol benzoate (Progynon-B)⁴ in sesame oil was injected intramuscularly in the daily dosages listed in table 2. Dog RG received a total of 23.4 mgm. in 14 days; dog S, 30.0 mgm. in 13 days; dog P, 21.7 mgm. in 13 days. Dog F, in which hourly excretion studies were not made, and which does not appear in the table, received 0.123 mgm./kgm. for 11 days (total, 18.3 mgm.).

² Three cubic centimeter aliquots of plasma and urine were added to 17 cc. of 3.0 per cent HPO_3 as soon as obtained. Protein precipitation was followed by centrifuging and filtering, and urines were further diluted with acid before colorimetric analysis. The high dilution needed for the urines eliminated the action of other reducing substances and allowed them to be analyzed in the same manner as the plasma filtrates.

³ Daily rations of 200 to 250 grams were given of a mixture of the following proportions: 100 grams cracker meal; 30 grams skim milk; 10 grams brewer's yeast; 4 grams $\text{Ca}_3(\text{PO}_4)_2$; 3 grams of salt mixture (10 parts NaCl, 1 part Fe-citrate, and 4 parts Mg-citrate); and 5 cc. of cod liver oil.

⁴ Courtesy of Dr. Max Gilbert, Schering Corp.

RESULTS. A. *Urinary ascorbic acid excretion.* Changes in hourly excretion are summarized in table 2. For economy of space, the collection intervals have been divided into convenient periods of 4 or 5 days in length, and the values given represent the average of all determinations made during these periods.

B. *Tubular reabsorption.* Since the tubular reabsorption of ascorbic acid increases with the glomerular load up to saturation of the tubules, changes in

TABLE 2

Effect of estradiol benzoate on ascorbic acid excretion, water balance, fasting plasma ascorbic acid, and hematocrit in normal and castrate dogs

(All values are averages of determinations done during 4 or 5 day periods)

NUMBER OF DAYS IN PERIOD	ESTRADIOL INJECTED DAILY	ASCORBIC ACID EXCRETION	PLASMA ASCORBIC ACID	URINE FLOW	WATER INTAKE	HEMATOCRIT
Dog S (19 kgm.)						
	mgm./kgm.	mgm./hr.	mgm. per cent	cc./hr.	cc./hr.	per cent
5	Control	3.41	0.48	52.5	75.0	35.4
4	0.088	4.20	0.50	56.0	108.0	35.5
4	0.088	4.95	0.32	70.0	107.0	32.5
5	0.171	5.26	0.30	75.0	119.0	30.5
5		5.91		74.3	100.0	26.5
5		5.82	0.48	57.0	100.0	11.0
5		4.70	0.35	52.8	77.0	16.0
Dog RG (18 kgm.)						
2	Control	4.87	0.45	37.0		35.0
4	0.0925	5.18	0.31	31.2		42.0
5	0.0925	8.70	0.42	67.4		37.5
5	0.0925	7.72	0.32	79.0		36.2
5		6.61	0.30	41.0		31.2
5		4.34	0.35	52.2		28.0
5		4.93	0.33	36.0		30.5
Dog P (castrate) (14 kgm.)						
4	Control	4.77	0.39	27.7	43.0	35.0
5	0.075	3.55	0.37	24.8	39.0	43.0
4	0.087	4.37	0.36	24.4	41.2	44.0
4	0.111	4.59	0.30	22.4	38.0	39.0
4	0.111	5.67	0.32	23.0	38.0	35.5
4		6.11	0.40	27.5	39.0	26.7

reabsorptive capacity resulting from estradiol treatment must be compared with normal values in terms of the load at which reabsorptive activity is measured. Accordingly, control and experimental data are presented in figure 1 in terms of T/T_m ratios as plotted against load/ T_m ratios, where T is the observed reabsorptive activity at a particular load and T_m is the maximal reabsorptive capacity, as independently measured at load values high enough to assure saturation. The control data on four dogs, expressed as T/T_m in relation to load/ T_m , have been combined to give a mean curve which is taken as showing

the normal relationship between load and reabsorptive activity. This relationship can be spoken of as the ascorbic acid "titration curve," in the sense in which Smith and his co-workers (10) have spoken of the glucose and diodrast "titration curves" in the human kidney.

It is apparent that considerable splay is present in this titration curve, as compared with the curve to be expected in the case of a substance completely reabsorbed by the tubules at all load levels up to tubular saturation. It is also apparent that a load/ T_m ratio of over 2.5 is required to obtain complete saturation.

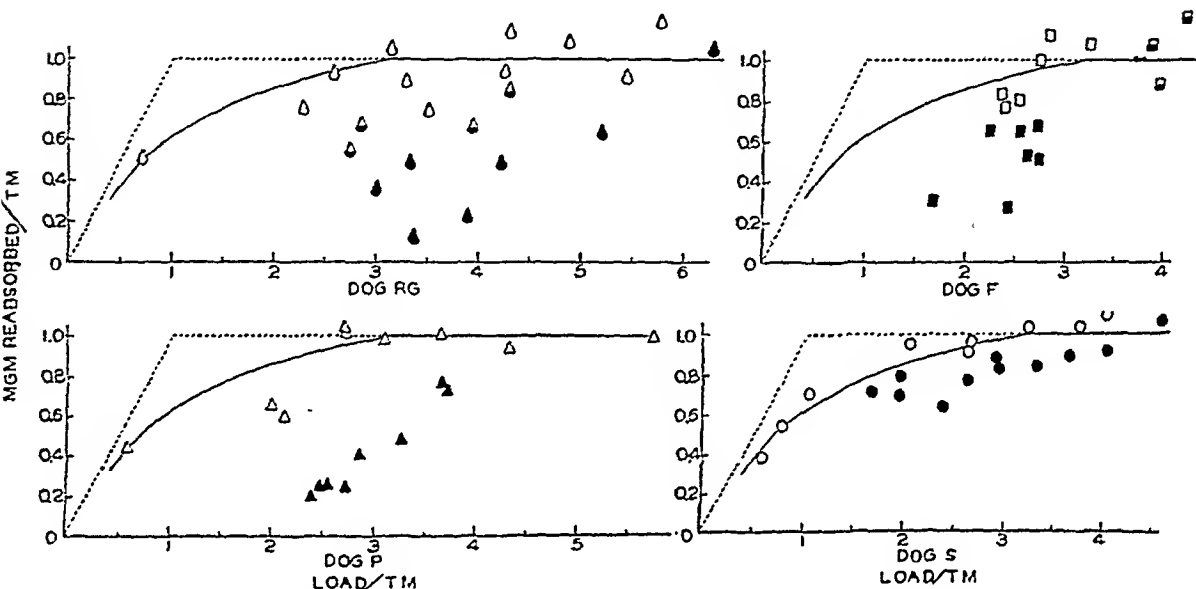


Fig. 1. Effect of estradiol benzoate on ascorbic acid reabsorptive capacity, as expressed in terms of T/T_m plotted against load/ T_m , where T is the observed reabsorptive capacity at any particular load, and T_m is the maximal reabsorptive capacity independently measured at load values high enough to assure saturation. Open symbols, control; solid symbols, during estradiol treatment; semi-solid symbols, recovery. Each symbol represents a clearance experiment into which are averaged three to six consecutive urine collection periods.

Control data have been combined in a mean "titration" curve which is taken to show the normal relationship between load and reabsorptive capacity. This curve was obtained by approximation of T/T_m ratios averaged in blocks of load/ T_m ratios. The dotted line shows the relationship to be expected for a substance completely reabsorbed by the tubules up to tubular saturation.

Because the effects of treatment differed in the various dogs, each is shown separately in figure 1, in reference to the above mean normal curve. T_m values during estradiol treatment are summarized in table 3, and in figure 1 it will be seen that in the treated animals reabsorption is reduced at loads which normally effect tubular saturation.

The decrease in tubular reabsorption increases the plasma clearance of ascorbic acid so that in some cases it approaches the creatinine clearance (fig. 2). The increased clearance tends to reduce the fasting plasma level of ascorbic acid during treatment (table 2).

C. *Glomerular filtration rate.* Increases of 13, 18, and 23 per cent in the average glomerular filtration rate were noted in dogs F, RG, and S, respectively. These changes may have resulted from expansion of the plasma volume, as indicated by a greater water intake proportional to urinary output early in the treatment (see dog S, table 2).

D. *Urine pH.* The control pH of the urine of dog F averaged 5.79 (25 observations), and in the experimental periods, 5.96 (28 observations). The absence of change in the urine pH during treatment indicates that this factor is not important in influencing the excretion of ascorbic acid in this study.

TABLE 3

The effect of the administration of estrogen on the ascorbic acid reabsorptive mechanism of the kidney in the dog

	Tm (MG./MIN.)			
	Dog F	Dog RG	Dog S	Dog P (castrate)
Control				
Range.....	0.485-0.671 (4)*	0.410-0.655 (9)	0.538-0.645 (6)	0.533-0.595 (6)
Average.....	0.600	0.542	0.590	0.571
During treatment				
Range.....	0.159-0.405 (5)	0.063-0.575 (7)	0.373-0.623 (10)	0.110-0.436 (8)
Average.....	0.314	0.262	0.488	0.235
After treatment				
Range.....	0.529-0.728 (3)	0.300-0.460 (4)	†	‡
Average.....	0.635	0.373		
Per cent decrease during treatment.....	48.0	52.0	17.0	59.0

* Numbers in parentheses denote the number of clearance observations entering into the average Tm. Each clearance observation consisted of from three to six continuous urine collection periods.

† Recovery not followed.

‡ Dog sacrificed before recovery.

E. *Creatinine and mannitol clearance.* While estradiol was being given, the creatinine/mannitol clearance ratio averaged 1.03 for 16 urine collection periods. Thirteen periods ranged from 0.97 to 1.03, but three (all during one series of clearance determinations) were unaccountably high, i.e., as high as 1.17.

F. *Blood and vascular changes.* Other workers (11) have reported in detail certain changes which we have noted. All dogs showed increased capillary fragility and petechial hemorrhages, and the clotting time was noticeably increased. Severe anemia resulted in the death of two of our dogs, F and S, several weeks after the injections were stopped.

G. *Kidney histology.* Kidneys were obtained from dog S twenty days after the last injection of estradiol, when she died from anemia. Except for post-mortem changes, sections of these kidneys appeared normal. A kidney was removed from dog P under anesthesia while receiving a daily injection of 0.111 mgm./kgm. of estradiol and during a maximal effect on ascorbic acid excretion and tubular reabsorption. Sections of this kidney also appeared normal.⁵

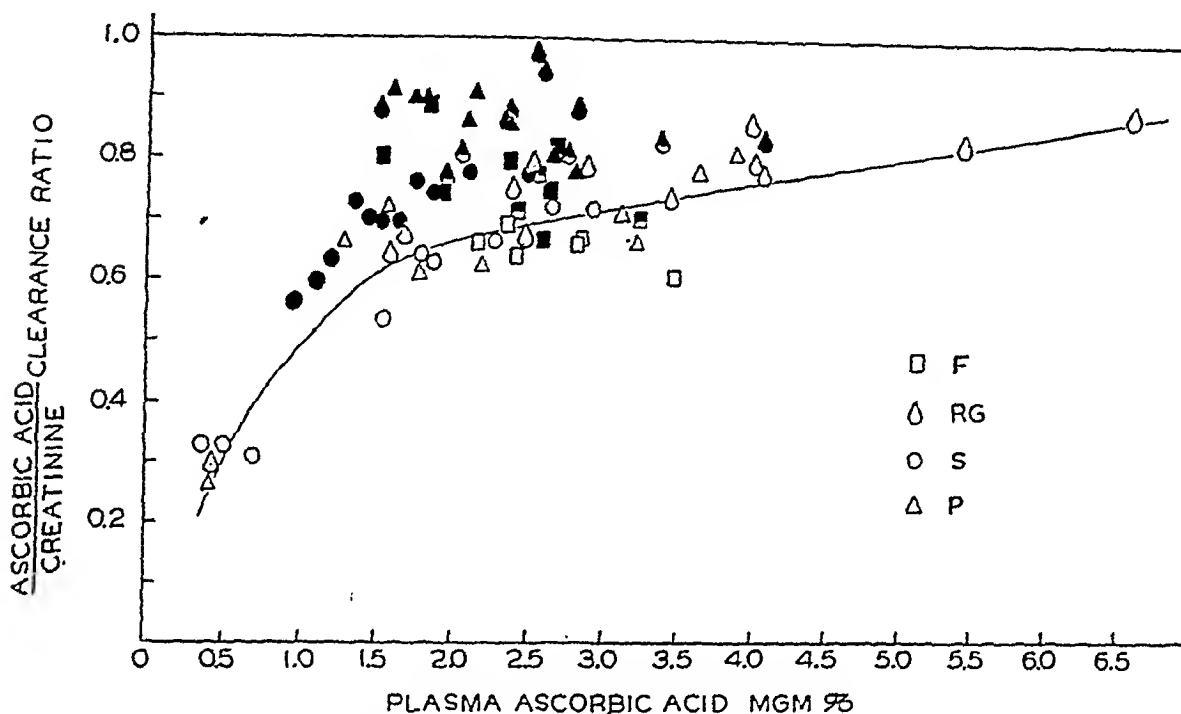


Fig. 2. Effect of estradiol benzoate on the renal plasma clearance of ascorbic acid at various plasma levels, as expressed in terms of ascorbic acid/creatinine clearance ratios. Open symbols, control; solid symbols, during estradiol treatment; semi-solid symbols, recovery. Each symbol represents a clearance experiment into which are averaged three to six consecutive urine collection periods.

The mean normal curve was obtained by approximation of clearance ratios averaged in blocks of plasma ascorbic acid concentration.

DISCUSSION. The increased urinary excretion of ascorbic acid following injections of estradiol benzoate in the dog appears to be due to a decreased renal tubular reabsorption of this substance. The decreased tubular reabsorption increases the renal plasma clearance so that in some cases the latter approaches the creatinine clearance (fig. 2). Hence depletion of plasma ascorbic acid occurs, and this presumably leads to acceleration of ascorbic acid synthesis, thereby further increasing the rate of excretion through maintenance of the plasma level.

⁵ The only significant alteration noted in the kidney of dog P concerned the renal arterioles, which were universally thick and with small lumina. The cells of the media appeared large, fairly clear, and sometimes in two layers. The so-called juxta-glomerular apparatus was prominent whenever encountered; no granules were seen in its cells. In some areas there was a slight increase in interstitial connective tissue surrounding a few tubules and more rarely the glomerulus.

It is interesting to note that considerable ascorbic acid is excreted by the dog at low plasma levels, indicating that tubular reabsorption is never complete, a phenomenon also evident in man (12). Incomplete tubular reabsorption with increasing load levels is reflected in the splay in the ascorbic acid titration curve (fig. 1). Smith and associates (10) report a slight splay in the glucose titration curve of the human kidney, and on the explicit premise that glucose reabsorption is complete in each nephron up to the load at which saturation occurs, accept this splay as reflecting variations in the ratio of *filtration rate/glucose reabsorptive capacity* in individual nephrons. The splay in the ascorbic acid titration curve may thus reflect a disparity in the ratio: *filtration rate/ascorbic acid reabsorptive capacity* in individual nephrons. On the other hand, such a splay may reflect an intrinsic characteristic of the reabsorptive activity of the tubule cells with respect to ascorbic acid, whereby the efficiency with which ascorbic acid is removed from the tubular urine decreases as its concentration in the urine is increased. A choice between these alternatives cannot be made from the present evidence.

The fact that the splay appears to be so markedly accentuated by estradiol treatment, without any large change in the total filtration rate, suggests that estradiol affects the kinetics of the tubular reabsorptive process rather than by significantly altering the ratio of *filtration rate/ascorbic acid reabsorptive capacity*. This inference is supported by the fact that T_m is unchanged by treatment, although a greater load/ T_m ratio is required to effect saturation. It is further supported by the fact that the changes in the titration curve are not accompanied by histological changes in the renal tubules, and by the rapid reversibility of the changes in reabsorptive activity.

The means by which estradiol alters the tubular mechanism cannot at present be answered. One of the most striking changes seen in treated dogs is a marked depression of the hematocrit. However, the renal effect in all of our dogs occurred before the hematocrit had been greatly decreased, and tended to return to normal after treatment, when the hematocrit was invariably low. It therefore does not appear that anemia itself affects the renal mechanism.

Petechial hemorrhages and tendency to bleed after slight trauma were noted in all of the treated dogs. This apparent increase in capillary fragility may well be the result of enhanced ascorbic acid loss from the body, brought about by the altered renal mechanism which increases the plasma clearance of this substance.

SUMMARY AND CONCLUSIONS

1. In the normal dog, tubular reabsorption of ascorbic acid is incomplete at plasma levels considerably below those required to effect complete saturation of the tubules. Consequently, ascorbic acid is excreted in the urine at all plasma levels.

2. By reducing tubular reabsorption, estradiol benzoate increases the clearance of ascorbic acid. As a result, the rate of excretion is increased while the plasma level tends to be reduced.

3. During estradiol benzoate treatment, load levels considerably higher than those normally needed for tubular saturation produced a maximal rate of reabsorption equivalent to the normal. In the absence of any large change in glomerular filtration rate and with no significant histological changes, this was taken to mean that estradiol affects the kinetics of ascorbic acid reabsorption, rather than by changing the ratio of *filtration rate/reabsorptive capacity*.

4. Changes in tubular reabsorption are not attributable to changes in urine pH, decreases in hematocrit, or visible tubular damage.

5. Changes in water balance and slight increases in glomerular filtration rate were observed in normal dogs after estrogenic treatment, but not in one castrate. However, all showed a comparable alteration of the renal ascorbic acid mechanism.

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NEUROMUSCULAR TRANSMISSION IN THE SINGLE NERVE AND MUSCLE FIBER PREPARATION

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Within the past few years evidence has accumulated which supports the view that acetylcholine is the chemical transmitter of nervous impulses at the neuromuscular junction. The theory of chemical transmission has been based thus far on the results obtained from the whole muscle with its nerve supply. As yet, no systematic study has been made on the intact, innervated, single muscle fiber. It appeared desirable, therefore, to attempt this study.

METHODS. Frogs (*R. pipiens*) were used. The sartorius with its nerve supply was isolated and kept in modified Ringer (NaCl, 0.67 gram; KCl, 0.02 gram; CaCl₂, 0.02; gum acacia, 0.01; NaHCO₃, 0.02 gram; glucose, 0.02 gram; H₂O, 100 cc.). The nerve-sartorius preparation was then placed in a shallow pool of Ringer on a glass plate and the muscle, with its dorsal surface uppermost, fixed at both ends. The preparation was transilluminated and with the aid of a dissecting microscope a single muscle fiber was isolated. The technique used was a modification of the method described by Kato (1934). First, layers of muscle fibers were carefully peeled off with a fine iridectomy knife until only two or three layers remained. The muscle was then turned over with its ventral surface uppermost. In this aspect, the courses of the nerve branches could be seen readily. The nerve was stimulated with induction shocks to determine a group of active muscle fibers. All nerve twigs were then cut except the one going to these fibers. The latter were then teased apart at the upper pelvic end of the muscle. Test shocks were frequently applied to the nerve so that the active fibers could be identified. After one had been chosen, the others were cut. These fibers were next cut away on either side of the entrance of the nerve twig to the active fiber. The small remnants of muscle tissue (about 3 to 5 mm.), however, did not respond to either direct or indirect stimulation. No attempt was made to isolate the entire length of the fiber. At the pelvic and tibial ends a small number of cut muscle fibers were left and these helped to fix the active intact fiber. The remnants at either end also did not respond to stimulation since all nerve branches innervating them were cut.

The preparation was next placed in a Petrie dish filled with paraffin in which two small troughs at right angles had been made. The muscle fiber was placed in one trough and in the other the nerve lay across two small silver electrodes. The muscle fiber was put under a small amount of tension and both ends fixed. The responses of the fiber were recorded by the mercury droplet method of Pratt and Eisenberger (1919). The fiber was sprayed with fine mercury globules and the preparation then placed under a microscope. A globule was strongly

¹ National Research Council Fellow in the Medical Sciences.

illuminated and a demonstration ocular projected the reflected ray of light from the droplet onto a moving film. The muscle fiber could be visualized directly through the regular eye-piece of the microscope.

In some experiments a single muscle fiber from the retrolingual membrane of the frog was used. The preparation was made according to the method of Pratt and Reid (1930). A nerve filament was found and traced to a muscle fiber. The former was cut proximally and all other nerve filaments that were found were severed. In addition, all other muscle fibers in the vicinity of the one that was being recorded were also cut. "Unipolar" stimulation was used to activate the nerve. One electrode was a quartz-covered platinum wire (about 5μ diameter) placed on a micromanipulator, and the other was a large indifferent electrode placed in the bath covering the preparation. That the nerve was stimulated and not the muscle fiber was determined by placing the electrode close to the muscle fiber and stimulating it with the same strength of stimulus effective for the nerve. No response was obtained as the threshold for the muscle fiber was higher than that of the nerve filament.

The nerve was stimulated supra-maximally at various frequencies with condenser discharges. The drugs employed were acetylcholine chloride (Merck), eserine salicylate (Sharpe and Dohme), and curare (the crude drug).

RESULTS. I. Fatigue. It is well known that in stimulating a nerve-muscle preparation indirectly with tetanizing currents fatigue occurs first at the neuromyal junction, since the nerve can still conduct impulses and direct stimulation of the muscle elicits a contraction. Asmussen (1934) stimulated small bundles of muscle fibers indirectly and found that, with the onset of fatigue, the tension curve fell in a stepwise manner. That this effect was not due to fatigue of the muscle fibers themselves was shown by the response obtained to direct stimulation. Moreover, direct stimulation of a similar small bundle of muscle fibers resulted in a tension curve which fell smoothly with the onset of fatigue. On the basis of these results Asmussen concluded that the step-like fall of tension during indirect stimulation was due to an all-or-none behavior of the end-plate. Accordingly, it appeared desirable to test this conclusion directly with a single motor end-plate.

Figure 1 illustrates the results obtained in a typical experiment. A single muscle fiber was continuously stimulated indirectly at the rate of 5 per second. There was a progressive decline in the height of contraction (cf. A and B). However, the fiber responded to each stimulus for 1 minute 16 seconds after the start of stimulation. The fiber then suddenly failed to respond to a stimulus but did contract to the next eight stimuli when it again suddenly failed to contract. It will be observed from the subsequent sections of the record (C to F) that, at first, the failure of the muscle fiber to respond occurred infrequently. The interval between responses became progressively longer and after 5 minutes of stimulation a period of 12 seconds or more was noted between two successive responses.

When the fiber first began to fail to respond there was little change in the height of contraction, but as this failure became more frequent and more pro-

longed there was a progressive increase in the height of response until finally it approached that obtained at the start of stimulation (fig. 1A and F). A stimulus well above threshold was used throughout these experiments and at no time during the period of stimulation did a further increase in strength of stimulus have any effect on the responses of the muscle fiber.

Indirect stimulation of a single muscle fiber with frequencies between 100 and 155 per second produced, initially, a tetanus for 1 to 2 seconds followed by a fall in tension. There then appeared single responses at irregular intervals which became longer as stimulation continued. However, with these higher frequencies the responses soon ceased and did not return even though stimulation was continued from 1 to 5 minutes longer. Increase of strength of stimulus was also without effect. That the muscle fiber itself was not fatigued was shown by eliciting a contraction to a direct stimulus at varying times after the responses to indirect stimulation had stopped.

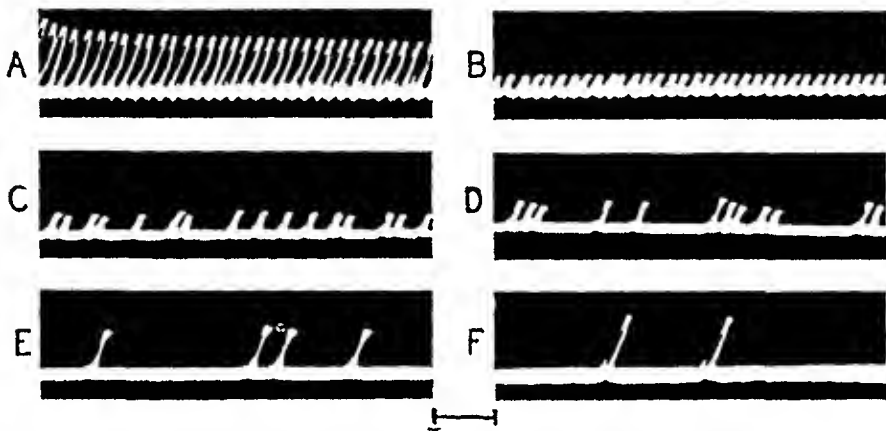


Fig. 1. Fatigue. Mechanical responses of a single muscle fiber stimulated continuously via its nerve at the rate of 5 per sec. A: beginning of stimulation; B to F: 1, 2, 2.5, 3.5, and 4.25 minutes later, respectively.

Direct stimulation of a single muscle with frequencies between 5 and 10 per second did not result in any sudden failure to respond as observed with indirect stimulation. The fiber responded to each stimulus and with the onset of fatigue there was a continuous decrement in the height of contraction (see Asmussen, 1934). This smooth decline in tension continued until the fiber was completely fatigued at which time there was no further response. With continued stimulation there was no return of the contraction. Here again increase of the stimulus, which was initially well above the threshold for the fiber, was also without effect. In some cases a nerve-muscle fiber preparation was used and the muscle fiber directly stimulated until it was completely fatigued and did not contract. At this point the muscle fiber was stimulated through its nerve but this also failed to produce a response. That the nerve fiber and neuromyal junction were intact in these experiments was shown by allowing the muscle fiber to recover, after which indirect stimulation resulted in contraction.

The results obtained from stimulating indirectly 2 to 4 muscle fibers confirm those obtained by Asmussen (1934). In a typical experiment 2 muscle fibers

were stimulated indirectly at the rate of 4 per second. The general curve followed that of figure 1. However, both fibers did not fail to respond at the same time. Instead, one fiber dropped out suddenly and the height of response was about half of the previous contraction involving the two fibers. The fiber would then respond intermittently. Soon the other fiber began to fail to contract and at this time there appeared intervals during which there was no response to some of the stimuli. When a contraction did occur it consisted of either one or both fibers.

This step-like effect was even more strikingly brought out by stimulating 4 muscle fibers indirectly. At first all the fibers contracted but with further stimulation each fiber began to drop out suddenly and then also responded intermittently. Four step-like gradations could be observed on the record. At times a stimulus failed to produce a response of any of the 4 fibers while the next effective stimulus might result in a response of any or all of the muscle fibers. The intervals between these contractions were not as long as those seen with the single muscle fiber as at least one of the fibers would soon respond.

II. *Eserine*. Most workers have been unable to observe any potentiation of the muscular response to single nerve volleys by eserine in amphibia. Thus

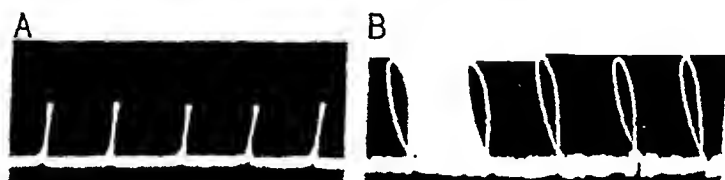


Fig. 2. Eserine. Single muscle fiber stimulated indirectly every 10 sec. A: before eserine. B: 5 min. after the addition of 0.3 mgm. eserine to the Ringer solution bathing the preparation.

Kruta (1935) employing eserine, and Cowan (1936) using prostigmine, reported negative results. Brown (1937b) also found that eserine (1 to 100 γ) perfused through the vessels of the isolated frog's leg or delivered in the circulating blood produced no augmentation of the response. However, Feng (1937) was able to obtain potentiation to single shocks in the isolated nerve-sartorius preparation and Hodes and Steiman (1939) confirmed this potentiation effect by injecting eserine into the dorsal lymph sac.

In the present experiments it was also found that eserine would potentiate the response of a single muscle fiber indirectly stimulated once every 10 seconds (fig. 2). Doses of eserine which ranged from 0.1 to 0.3 mgm. were added to the Ringer solution bathing the preparation. In all the preparations tested (8) there was approximately a 100 per cent increase in the height of contraction (cf. A and B, fig. 2).

With faster frequencies of stimulation (2 to 10 per sec.) after eserine, the fiber responded to the first few stimuli and then suddenly ceased to contract although stimulation was continued from one to two minutes or more. Frequently, the fiber again contracted after it had failed to respond to one or two stimuli. Occasionally there appeared, 10 to 15 seconds after the last response, another contraction and none after that.

III. *Acetylcholine and curare.* Dale, Feldberg and Vogt (1936) showed that acetylcholine is released at neuromuscular junctions on stimulation of the motor nerve. Moreover, in normal mammalian muscle, close arterial injections of acetylcholine produce a response that can be identified as a true propagated contraction by the accompanying outburst of action potentials (Brown, Dale and Feldberg, 1936; Brown, 1937a). However, an excess of acetylcholine will paralyze muscles instead of exciting them (Dale, Feldberg and Vogt, 1936; Rosenblueth, Lindsley and Morison, 1936). This fact, together with other experimental results, led Rosenblueth and Morison (1937) to postulate a paralytic level of acetylcholine. On the basis of the all-or-none law it should be expected, therefore, that as soon as the paralytic threshold is reached the single muscle fiber should suddenly cease to contract. That this is actually the case can be seen in figure 3. A single muscle fiber was indirectly stimulated every 2 seconds and gave the characteristic all-or-none response. After addition of 30 γ acetylcholine to the Ringer solution the fiber continued to contract until the diffusion



Fig. 3. Acetylcholine. Single fiber stimulated indirectly every 2 sec. at arrow 30 γ of acetylcholine were added to the bathing solution.

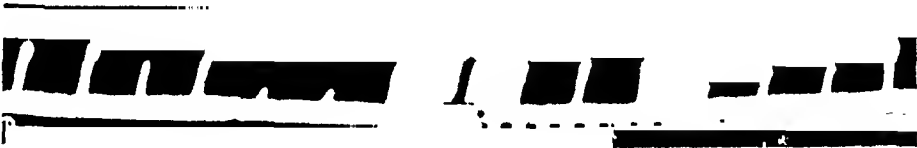


Fig. 4. As in figure 3, but records from 4 muscle fibers. At each arrow 15 γ of acetylcholine were added.

of acetylcholine reached a paralytic concentration at the end-plate and the fiber failed to respond to the next stimulus. A subsequent stimulus again elicited a contraction and there was no further response for 20 seconds. At the end of this time the contractions reappeared and these were of the same magnitude as those shown in the record.

Figure 4 illustrates the behavior of 4 muscle fibers to excess acetylcholine. A paralytic concentration was not reached at the 4 end-plates simultaneously and so there occurred a step-like gradation of the response as the individual fibers dropped out. In the record there are 4 distinct gradations of the total response, indicating further that each muscle fiber dropped out suddenly.

The all-or-none behavior of the motor end-plate is again demonstrated by the action of curare (fig. 5). After the addition of curare the responses continued until a concentration sufficient to block transmission was reached when the muscle fiber suddenly failed to respond. Although stimulation was continued for 2 min. or more there was no further contraction. Direct stimulation, however, was effective.

Acetylcholine has been shown to have a decurarizing effect on mammalian muscle (Briscoe, 1936; Rosenblueth, Lindsley and Morison, 1936). Hodes and Steiman (1939) were unable to demonstrate the decurarization effect of acetylcholine in the frog except in a few cases. In the present study the writer also was unable, in most instances, to obtain a decurarization by acetylcholine. However, in a few experiments, doses of 15 to 50 γ acetylcholine added after the fiber was curarized resulted in one or two contractions after which there were no more responses. Attempts to repeat this result by adding more acetylcholine to the Ringer's were unsuccessful. It appeared that the optimal condition for obtaining the decurarization effect was a degree of curarization where a stimulus just failed to produce a response. The exact dose of acetylcholine was difficult to determine for a given preparation and the stage of curarization where a contraction would result could be easily missed.

DISCUSSION. The results obtained on single-fiber nerve-muscle preparations (figs. 1 to 5) confirm the results and inferences of previous studies on large groups of fibers. The process of neuromuscular transmission follows the all-or-none law because the first step of muscular activity—the propagated disturbance—is itself quantal. When repetitive stimulation (fig. 1) or excess acetylcholine

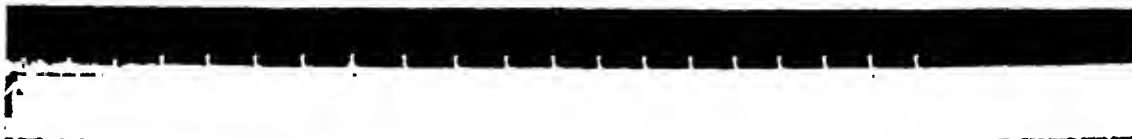


Fig. 5. Curare. Single muscle fiber stimulated indirectly every 2 sec. Curare was added at the beginning of the record (arrow).

(figs. 3 and 4) or curare (fig. 5) leads to a failure of transmission this failure is sudden in the single fiber.

The data on fatigue (fig. 1) support the distinction made in previous studies from this laboratory (Rosenblueth and Luco, 1939; del Pozo, 1942) between transmission and contraction fatigue. Thus, the decrease of response from A to B in figure 1 corresponds to contraction fatigue, since transmission has not failed. The increase of contractions from D to F, on the other hand, corresponds to a recovery from fatigue of the contractile systems, a recovery which occurs because the progressive development of transmission fatigue leads to relatively long rest intervals between the responses.

The random appearance of twitches in figure 1 C to F, after transmission fatigue first became manifest, suggests random changes in one or more of the factors involved in the process of transmission. These random variations have also been observed in whole muscle experiments both during the 4th stage (fatigue) and during the later 5th stage of relative recovery of transmission (Rosenblueth and Luco, 1939).

All the phenomena studied have been satisfactorily explained by the theory of neuromuscular transmission mediated by release of acetylcholine at the motor nerve endings.

Transmission fatigue (fig. 1) is due to a decrease of the quanta of acetylcholine per nerve impulse. Potentiation by eserine (fig. 2) is due to persistence of acetylcholine at the junction, protected by the eserine from the destruction promoted by cholinesterase. Block of transmission by acetylcholine (figs. 3 and 4) results from excess of the mediator. Block by curare (fig. 5) is due to the fact that the drug raises the threshold of muscle to acetylcholine. The experiments are not crucial, however, with respect to theories of transmission—i.e., other interpretations would cover the data.

SUMMARY

In a single muscle fiber indirectly stimulated at various frequencies transmission fatigue and contraction fatigue appear as independent phenomena (fig. 1).

The neuromuscular junction reacts in an all-or-none manner to fatigue (fig. 1), eserine (fig. 2), acetylcholine (figs. 3 and 4) and curare (fig. 5).

I wish to thank Dr. A. Rosenblueth and Dr. H. Davis for their helpful advice during the course of this study.

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THE KIDNEY AS A SOURCE OF GLUCOSE IN THE EVISCERATED RAT^{1,2}

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Bergman and Drury (1938) demonstrated that the presence of functional kidneys led to a decrease in the apparent rate of utilization of glucose by the eviscerated rabbit. The author (Reinecke, 1942) found that if no sugar were given, the non-nephrectomized, eviscerated rat would survive much longer than the nephrectomized, eviscerated rat. Since these animals probably die of hypoglycemia, this finding, too, suggested that the kidney was of importance in carbohydrate metabolism. In an effort to elucidate just what the rôle of the kidney may be, the experiments reported here were undertaken.

METHODS AND MATERIALS. Male albino rats obtained from Sprague-Dawley, Inc. were used. They were eviscerated when about 300 grams in weight, and were allowed to go without food for about 24 hours before being subjected to the operation. Prior to this they were maintained on tap-water and Purina "Whelping Fox Chow" or Alber's "Friskies" given ad libitum.

Evisceration. Since the operative procedures are of critical importance in these experiments, they are described in some detail. In order to obtain the best possible collateral circulation for the segment of vena cava that traverses the liver, this vessel is tied off just caudal to the liver in a preliminary operation performed when the rat is weaned or shortly thereafter. With the animal under ether anesthesia, the peritoneal cavity is opened by a ventral midline incision starting at the tip of the ensiform process and extending about 1.5 cm. caudally. The intestines are pushed to the animals' left and caudally by a flat instrument such as a scalpel handle. A thumb forceps with rather wide tips is then used as a double bladed retractor to maintain the exposure of the caudal portion of the vena cava where it enters the liver. A ligature of size "A" serum proof silk is passed through the thin mesentery suspending the liver from the dorsal surface of the peritoneal cavity at a point just slightly cranial to the entrance of the vena cava into the liver substance. This ligature is then used to tie off the vena cava at the level where it enters the liver. The incision in the abdominal wall is closed in two layers with size "A" silk. Usually the operative mortality is less than 5 per cent. If the ligature is placed in a more caudal position, the right kidney will often be found to be completely atrophic at the time of evisceration. Placing the ligature more caudally, furthermore,

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² The author wishes to express his appreciation for the encouragement and advice given to him by Dr. L. T. Samuels of the Division of Physiological Chemistry of the University of Minnesota.

tends to cause more bleeding with the consequent formation of troublesome adhesions. The formation of adhesions is also favored by the use of heavier silk or by excessive handling of the intestines such as would occur if they were temporarily removed from the abdominal cavity. Sterile procedure is unnecessary, but sulfonamides may be used with advantage in closing the abdominal wall. They should not, however, be placed in the peritoneal cavity for they tend to cause adhesions.

The evisceration proper is performed under ether anesthesia. The abdomen is opened along the midline ventrally from the base of the ensiform process to the level of the prevesical space. The intestine is retracted to the animal's right and the eoeliac and superior mesenteric arteries are exposed to their origins from the aorta by blunt dissection and are tied with a single ligature. (In about 5 per cent of the animals these arteries are found to arise as a common trunk.) The esophagus is then connected to the rectum by a 3-5 cm. length of no. 8 French rubber catheter that has been fitted with a glass cannula at either end. This procedure improves the viability of the preparation by preventing the overflow of mucus into the pharynx and its consequent interference with respiration. The cannulae are tied into place in such a manner as to prevent bleeding from the esophageal and colonic stumps. The caudal cannula is introduced into the descending colon through a rent made opposite the mesenteric attachment. It is advanced caudally until it is at the level at which the inferior mesenteric artery turns cranially to run alongside the colon. It is then tied in place with a ligature that also includes all of the vessels that lie parallel to the colon. The cranial cannula is introduced into the esophagus via a rent made in the stomach near the cardia. It is tied in place with a ligature that serves in addition to occlude the vessels found in the esophageal wall. At this stage in the operation, since its blood supply has been completely cut off, the section of the gastrointestinal tract intervening between the cannulae is removed along with the spleen and pancreas by severing the mesenteric attachments to the dorsal wall of the abdominal cavity and to the liver.

The next step is the removal of the liver. The mesentery attaching the liver to the dorsal abdominal wall is divided by blunt dissection and then a ligature is tied over the original ligature on the vena cava in such a way as to include the small adhesions that form after the preliminary operation and which tend to bleed slightly if not tied off. The falciform and triangular ligaments and the portion of the lesser omentum between the esophagus and liver are divided and a ligature is placed on the vena cava just cranial to the point at which it leaves the liver. The liver along with the portion of vena cava intervening between these ligatures is then removed. A button of liver weighing about 50 mgm. must be left on the cranial stump of the vena cava to prevent the movements of the diaphragm from slipping the ligature off the stump of the vena cava. It is extremely unlikely that this bit of liver exerts any influence on the fate of the preparation for not only is it small but also it is separated from the circulating blood by a ligature.

If the kidneys are to be removed, a noose of silk is slipped over each and tied

tightly around the vessels. The kidneys can then be dissected away with a minimal loss of blood. Care is taken to leave the adrenal glands as intact as possible. If on the other hand the adrenals are to be removed, they are similarly tied off and removed along with their enveloping adipose tissue.

The operative procedures are completed by closing the abdominal wall in a single layer. Size "C" serum proof silk is a convenient size and type of material to use for ligatures and suture material throughout. With practice the operation should be performed in 15 to 25 minutes.

Analytical procedures. The blood sugar determinations unless otherwise specified were carried out by a micro ferricyanide method using the filtrate from 0.02 cc. of blood from which the proteins were precipitated by tungstic acid (Reinecke, 1942b). When micro fermentation is to be used in conjunction with this method to determine the fermentable reducing substance in blood, 0.05 cc. of blood is measured into the 5 cc. of the dilute tungstic acid reagent rather than the 0.02 cc. used otherwise. Two cubic centimeters of the filtrate obtained is diluted with 3 cc. of distilled water. The blood sugar determination is then carried out on this diluted, unfermented filtrate in the usual way. The conventional blood sugar value thus obtained represents the sum of the fermentable and non-fermentable reducing substances in the blood that are not removed by the treatment with the tungstic acid reagent.

The remainder of the undiluted filtrate from the 0.05 cc. sample of blood is fermented with baker's yeast to give the glucose equivalent of the non-fermentable reducing substance. Fleischmann's yeast of the type sold in 1 lb. bricks is washed thoroughly with water. One part of yeast is suspended in four parts of water by vigorous stirring. The yeast is then centrifuged down and the water decanted. This is repeated ten times. The yeast is then made up in a 20 per cent suspension with distilled water and is used within an hour or two. The suspension is measured in 0.5 cc. portions into 11 x 60 mm. culture tubes which are then centrifuged at high speed. The supernatant water is removed with a fine glass capillary attached to a suction pump. The remaining filtrate, which amounts to about 2.5 cc., is poured onto the yeast. The yeast is resuspended by vigorous shaking. A square of "Parafilm" held in place with the finger tip may be used to close the open end of the tube. The yeast is then incubated with the filtrate for about 45 minutes in a water-bath held at 30°C. The tubes are shaken to resuspend the yeast about twice during this period. Then the yeast is again centrifuged down at high speed. Two cubic centimeters of the supernatant fluid are carefully removed with a pipet and diluted with 3 cc. of distilled water. The glucose equivalent of the non-fermentable reducing substance is then determined in this diluted, fermented filtrate. This value is corrected for the amount of reducing substance contributed by the yeast itself by subtracting the value obtained from a blank made up in a comparable manner from distilled water that had been incubated with a portion of the yeast concurrently with the filtrate. This blank usually gives a value equivalent to less than 10 mgm. per cent of glucose in the original blood. The difference between this corrected glucose equivalent of the non-fermentable

reducing substance and the conventional blood sugar value is taken as the glucose equivalent of the fermentable reducing substance in the blood. Under the circumstances of the experiment it is probable that this was largely glucose. As an additional check on the procedure, it has been our custom to ferment solutions containing glucose in excess of that to be found in the filtrates, concurrently with the filtrates. No significant amounts of sugar have remained in these solutions.

RESULTS. *The influence of blood loss and the administration of fluid on the blood sugar levels and survival of the non-nephrectomized, eviscerated rat.* In as

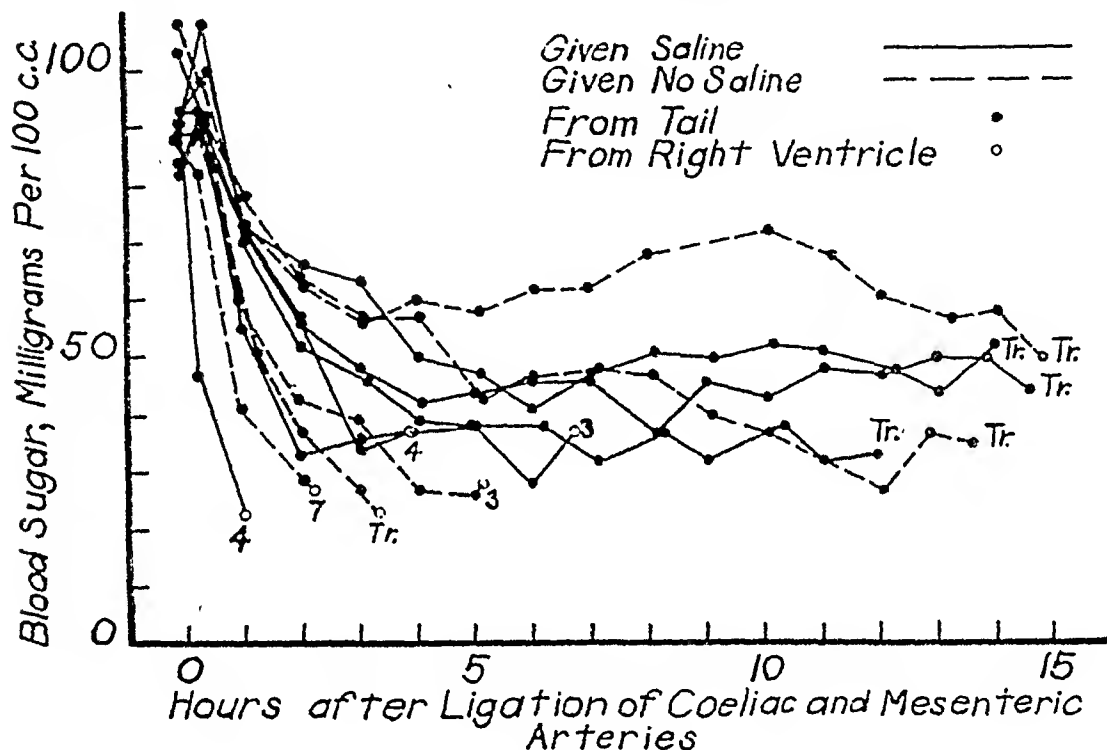


Fig. 1. *The influence of blood loss and the administration of fluid on the blood sugar levels and survival of the non-nephrectomized, eviscerated rat.* The animals given physiological saline received 2 cc. subcutaneously and 1 cc. intravenously (via the venous spaces of the penis) per 100 grams of body weight. The solution was given immediately after the completion of the operative procedures. The number shown near the final plotted value for each animal represents the approximate blood loss in grams. Tr. indicates that only traces of blood were lost. All the animals were eviscerated within a period of five hours.

much as Bergman and Drury had found that only the kidneys that were actively secreting urine were effective in reducing the apparent glucose consumption of the eviscerated rabbit, this experiment was planned to determine if the administration of physiological salt solution immediately after the evisceration would act as a diuretic and perchance improve the viability of the preparation. Quite accidentally during and after the eviscerations, several of the animals suffered an appreciable loss of blood. This was estimated by weighing the bits of cotton used to sponge up the shed blood. The results as presented in figure 1 indicate that loss of a few cubic centimeters of blood markedly reduces the survival of

this preparation and that, surprisingly enough, it seems that the cause of death is hypoglycemia. This is suggested not only by the low sugar levels found at death but also by the fact that all of the animals dying before seven hours after evisceration exhibited convulsions. This type of convulsion in the previous experience of the author has always been relieved by the injection of glucose.

The concentration of sugar in the blood at first decreased and then remained nearly constant in those animals that survived for ten or more hours. Unfortunately it was impossible to observe all of the animals continuously until they died, but two were alive 21 hours after they had been eviscerated. At this time their blood sugar levels were still within the range at which they had stabilized a few hours after evisceration.

All of the animals that survived beyond ten hours passed urine on one or more occasions after evisceration. The shorter lived animals did not void and had little or no urine in their bladders at death. The significance of this, however, is difficult to evaluate for, while this decrease in urine formation may have been due to impaired kidney function, yet it may have been due to the fact that these shorter lived animals just did not live long enough to secrete much urine.

The influence of fluid administration, for the determination of which the experiment was originally designed, seems to have been rather minor. Only one of the animals that had been given no fluid and had suffered only minimal blood loss showed the expected shorter survival period.

The effect of nephrectomy on the survival and blood sugar levels of the eviscerated rat. The results of a comparison of the non-nephrectomized eviscerated, with the nephrectomized eviscerated, rat are shown in figure 2. It was found that the blood sugar levels of the nephrectomized animals fell precipitously until the animals expired. In the case of the non-nephrectomized animals, however, the blood sugar levels fell to some low value that was still compatible with life and then stabilized there for many hours before the animals eventually died. The apparent blood sugar levels of the non-nephrectomized animals were higher at death than those of the nephrectomized animals. It must be recalled, however, that the method used for determining the blood sugar includes some non-fermentable reducing substances. The fact that animals in both groups exhibited convulsions before death strongly suggests that they died of hypoglycemia in both cases.

The rôle of the adrenal in maintaining the blood sugar in the eviscerated rat. Because nephrectomy is very likely to interfere with the normal function of the adrenal gland and because of the known importance of this gland in carbohydrate metabolism, adrenalectomized, eviscerated rats were compared with similar non-adrenalectomized animals. The results shown in figure 3A seem to indicate that the adrenal is not of major, immediate importance in maintaining the blood sugar concentration in the blood of the eviscerated rat. It is true that the adrenalectomized animals showed a greater decrease in blood sugar than did comparable non-adrenalectomized animals, but while it is possible that this

may be a specific effect, yet it is also likely that it may be incidental to some impairment of renal circulation due to the manipulations performed in adrenalectomy. The material presented in figure 3B is taken from the unpublished data of Reinecke and Kendall. It shows that the injection of adrenal cortical extract is without major acute effect on the blood sugar of the eviscerated rat. This result was also obtained by Selye and Dosne (1940), but the characteristics of the preparation used by these investigators were so different that it is doubtful if their data are comparable.

The effect of the kidney on the concentration of sugar in the blood flowing through it. The influence of a functioning kidney on the concentration of sugar in the

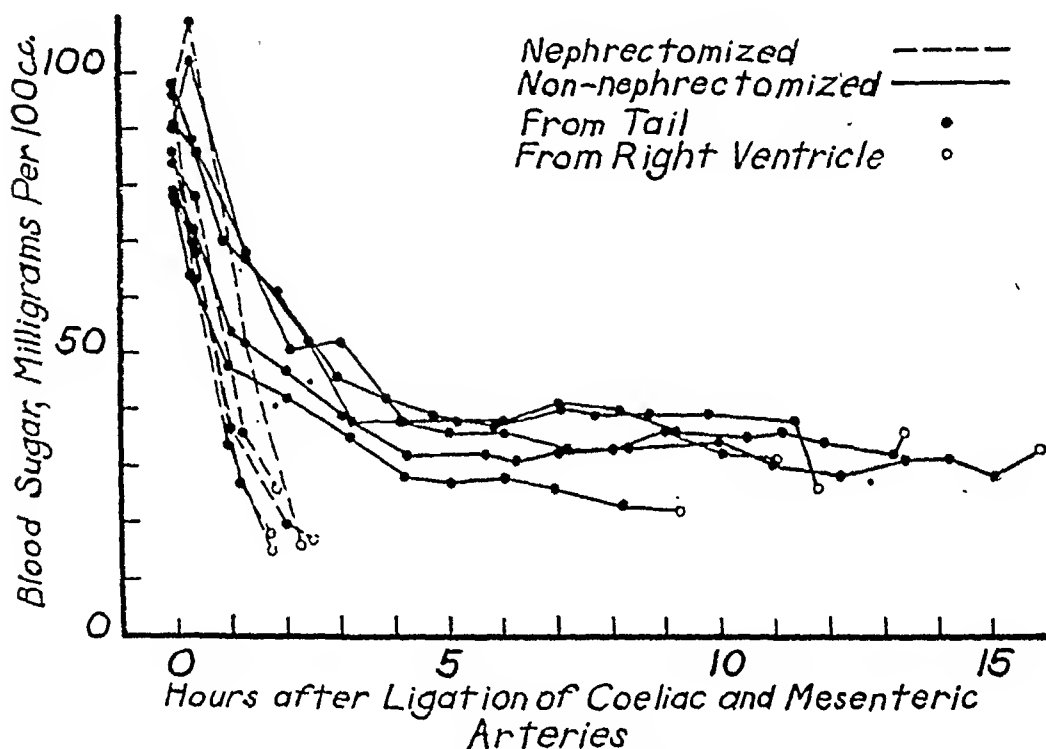


Fig. 2. The effect of nephrectomy on the survival and blood sugar level of the eviscerated rat. All animals were given 2 cc. of physiological saline per 100 grams of body weight at the time of evisceration. This was administered subcutaneously. All the eviscerations were completed within 7 hours.

blood of the eviscerated rat might possibly be explained in three ways: 1, the kidney itself may act as a source of blood sugar; 2, the presence of the kidney may allow other tissues to act as a source of blood sugar; 3, the presence of the kidney may lead to the utilization of glucose at a slower rate than occurs in its absence. The application of Occam's "Razor" suggests the first possibility. (Bergman and Drury, however, postulated the third alternative because they found that tying off the ureters without interfering with the blood vessels of the kidneys led to an apparent increase in the rate at which glucose was utilized. From this they argued that the kidneys were excreting some substance which stimulated glucose utilization.) A study of the sugar concentrations in the blood

of the aorta and renal vein of the eviscerated rat was therefore undertaken to determine whether or not the first possibility might afford an explanation. The results are shown in figure 4. The concentration of sugar-like reducing substance was consistently found to be higher in the renal vein blood and lower in the vena caval blood (with one exception that may be experimental error) than in the aortic blood. That these differences are due to actual differences in the amount

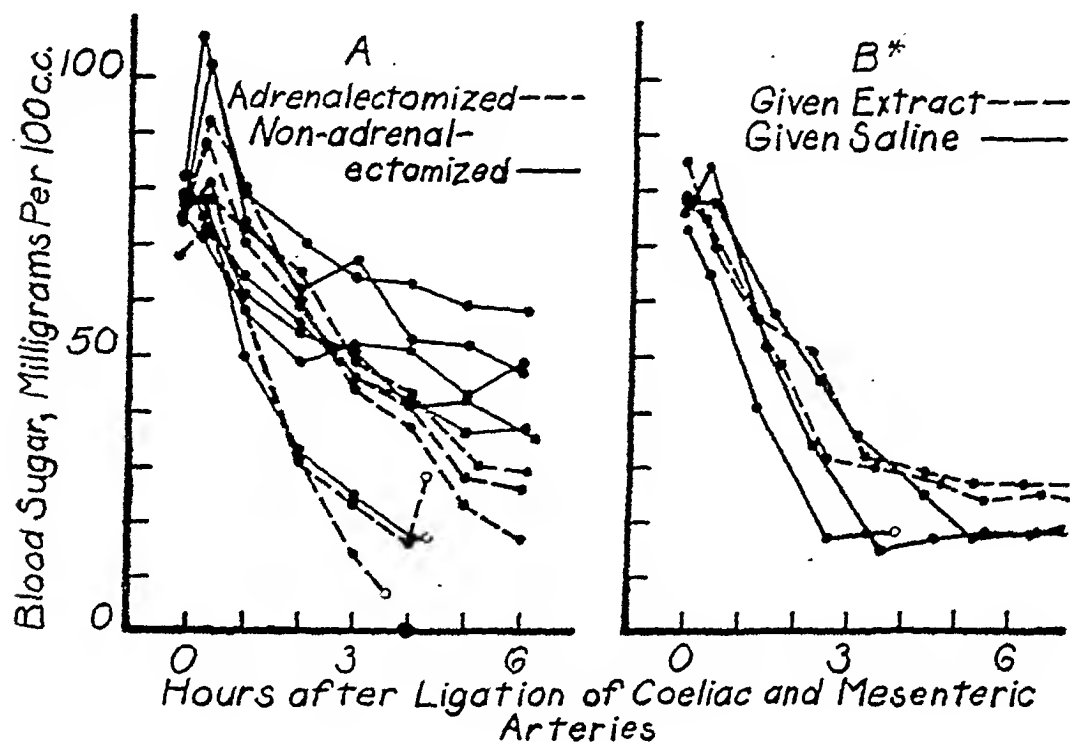


Fig. 3. The role of the adrenal in maintaining the blood sugar in the eviscerated rat. (A) All animals were given 2 cc. of physiological saline per 100 grams of body weight at the time of evisceration. This was administered subcutaneously. All the operative procedures were completed within a period of 5 hours. (B) These animals were slightly over 200 grams in weight at the time of evisceration. All were given 1 cc. of physiological saline per 100 grams of body weight and a dose of phenolsulfonphthalein intravenously shortly after evisceration; 5 cc. of an aqueous solution containing the extract of 75 grams of adrenal gland per cc. was administered subcutaneously immediately after the intravenous injection to the indicated animals. The others were given a corresponding amount of physiological saline in a similar fashion. The methods used were comparable in general to those described in this paper.

* These data are taken from some unpublished studies of R. M. Reinecke and E. C. Kendall of the Mayo Clinic, Rochester, Minnesota.

of glucose present, at least with respect to the renal vein and aortic blood, is strongly suggested by the fact that they were also apparent when the fermentable reducing substance was determined.

An incidental finding was that the concentration of reducing substance in the renal vein blood tends to increase in consecutive samples taken at short intervals.

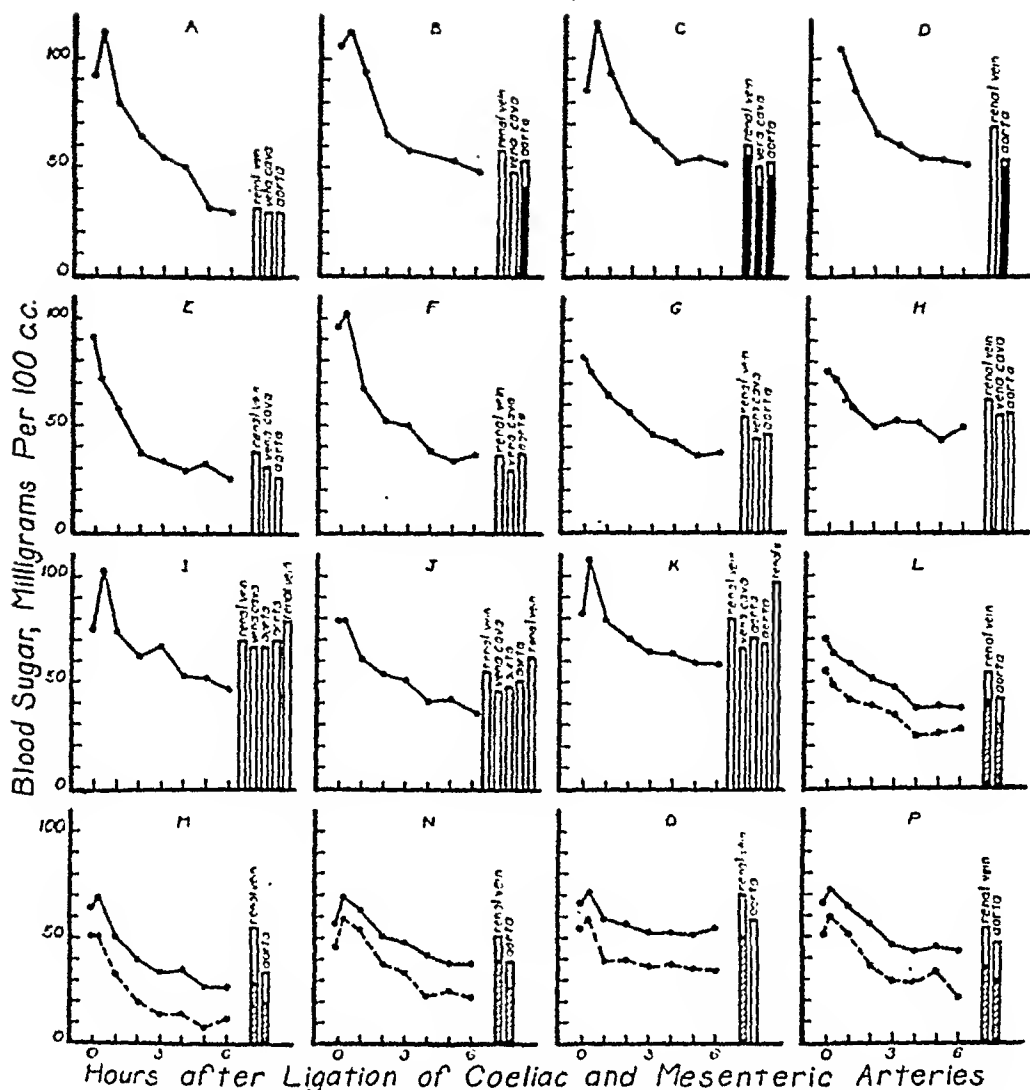


Fig. 4. The effect of the kidney on the concentration of sugar in the blood flowing through it. All animals were given 2 cc. of physiological saline per 100 grams of body weight subcutaneously at the time of evisceration. The arterial and venous samples were taken with the animals under anytal anesthesia (9 mgm. per 100 grams body weight were given subcutaneously). (A-F) Large samples, 0.5-1.0 cc., were drawn simultaneously from the vessels indicated. (G-K) Small samples were obtained by puncturing the indicated vessel with a no. 27 hypodermic needle and collecting the blood in a capillary pipet from the small pool that formed. Samples were taken consecutively in the order shown by the bars from left to right. (L-P) Small samples, 0.10-0.15 cc. were drawn simultaneously with 0.25 cc. hypodermic syringes.

The heights of the entire bars represent the glucose equivalent of the reducing substances in the tungstic acid filtrate of the blood samples as determined by the micro blood sugar method referred to. Solidly shaded portions correspond to the values obtained with the methods described by Somogyi (1930, 1937). The cross-hatched portions represent fermentable reducing substance determined in the manner described in this article. The solid-line curves represent glucose equivalents obtained in the same way as those represented by the heights of the entire bars. The broken-line curves like the cross-hatched portions of the bars represent fermentable reducing substance. The samples represented by the bars were taken within a few minutes after the last samples represented in the curves. The lack of shading or cross-hatching in the majority of the bars indicates that the corresponding determinations were not made in these instances.

The arteriovenous differences of the glucose equivalents of the fermentable reducing substance for L, M, N, and P were respectively 11, 9, 13, and 6 mgm. per cent.

The three groups, A-F G-K, and L-P were studied separately on three different days.

DISCUSSION. The increase in the concentration of sugar or sugar-like material in the renal vein blood over that found in the aorta is too large in most instances to be explained by the slight concentration of the blood due to the excretion of water by the kidney. Since the carbohydrate reserves of the kidney are small, it would therefore seem that there must be carbohydrate formation in this organ.

The increasing concentration of reducing substances in consecutive samples taken from the renal vein may reflect an increased mobilization of sugar by the kidney under the influence of the nervous stimulation occasioned by piercing the renal vein.

The addition of fermentable reducing substance to the blood by the kidney affords a ready explanation for the fact that non-nephrectomized, eviscerated rats were able to maintain a concentration of sugar in their blood that is compatible with life while comparable nephrectomized animals quickly succumb to hypoglycemia. In the light of the other findings it seems probable that the reason for the early deaths of the non-nephrectomized animals that have suffered blood loss lies in a failure of their kidneys to supply sugar to the blood stream.

The previous work of other investigators offers some support for the concept that the kidney is a source of blood sugar. This of course is one of the possible interpretations of the findings of Bergman and Drury which has already been mentioned. Goda (1938) and Stewart and Thompson (1941) showed that the kidney could convert fructose to glucose; this, however, is a substrate that is not likely to be present in significant quantities in the present instance. Russell and Wilhelmi (1941), however, have shown that the addition of some of the amino acids could increase the formation of glycogen in the kidney slice. The amino acids probably are available in the preparation used in these experiments and are one of the probable sources from which the kidney forms glucose.

It is of interest to note that Himsworth (1938) and Himsworth and McNair Scott (1938) obtained some evidence that there are extra-hepatic sources of blood sugar in the hepatectomized rabbit. The results of the experiments just described would of course suggest that the source of blood sugar may have been the kidneys.

SUMMARY

In the presence of the kidneys the concentration of sugar in the blood of the eviscerated rat at first falls but then remains at a nearly constant value for a period of many hours.

Loss of blood causes the prompt development of hypoglycemia in the non-nephrectomized, eviscerated rat.

The concentration of sugar in the blood of the nephrectomized, eviscerated rat falls precipitously until the animal dies.

Adrenalectomy or the administration of adrenal cortical hormones is without major, acute effect in the non-nephrectomized, eviscerated rat.

During the period in which the eviscerated rat is able to maintain the con-

centration of sugar in its blood at a nearly constant value, fermentable reducing substance is added to the blood as it flows through the kidneys.

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THE EFFECT OF SALINE WASHINGS OF ISOLATED JEJUNAL LOOPS ON GASTRIC SECRETION¹

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Studies of the effects of implanting pedicle grafts of jejunum into the wall of the stomach have revealed that the normal responses to such secretory stimulants as histamine and alcohol of increased acidity of the gastric juice and lowered pH of the gastric mucosa may be reversed or markedly diminished (1-4). When portions of duodenum, jejunum, ileum or colon are used for such implants the factor causing this alteration of gastric secretion is seen to be confined to the duodenum and jejunum, the latter source possessing somewhat greater activity (3). Further studies have indicated that while a gastro-enterostomy almost uniformly fails to produce this effect, it appears promptly after the bowel about the stoma is converted to a pedicle graft.

These findings have led us to postulate the presence in the lumen of the jejunum, and perhaps to a lesser extent in the duodenum, of a substance which, when introduced into the stomach, exerts a definite effect on the response of the acid secreting mechanism to certain secretagogues—notably histamine. The present report deals with the results of further efforts to demonstrate the presence of such a substance in isolated loops of jejunum.

METHODS. Healthy mongrel dogs (donors nos. 6-10, table 2) were operated upon and a segment of jejunum isolated as for a pedicle graft, but instead of its being opened and implanted in the wall of the stomach, one end of the segment was inverted under mattress sutures and the other end brought out through a stab wound at one side of the laparotomy incision. In three animals the segment was approximately 6 cm. in length, while in two it measured 12 to 15 cm. The continuity of the intestinal tract was re-established by end-to-end anastomosis.

After at least two weeks had elapsed to allow for healing, these loops were washed out two or three times with 25 cc. of normal saline and the washings collected.

To study the effects of such washings on gastric secretion the following technic

¹ This work was carried out under a grant from the John and Mary R. Markle Foundation.

was employed. Five normal dogs (recipients nos. 1-5, table 2) which had had at least one previous gastric analysis and had been trained to tolerate a small stomach tube were used as subjects, having been without food for 24 hours. After collecting the normal fasting secretion during a period of 10 minutes, the entire 25 cc. of jejunal washings was instilled into the stomach and allowed to remain for 10 minutes when it was removed, together with the gastric secretion during this period. At this point the dog received the usual 1 mgm. of histamine

TABLE 1
Effect of washings of isolated jejunal loops on gastric secretion
Control series

Gastric analyses following histamine in normal animals

RECIPIENT NO.	FASTING SPECIMEN			0-10 MINUTES			10-20 MINUTES		
	Vol.	Free acid	Total acid	Vol.	Free acid	Total acid	Vol.	Free acid	Total acid
	cc.			cc.			cc.		
1. N.....	11	18	34	13	34	44	13	94	106
S.....	16	66	82	3.5	35	45	19	56	72
2. N.....	11	22	50	14	46	80	15.6	76	104
S.....	9	28	52	31	68	84	23	86	96
3. N.....	11	26	42	6	22	42	14	64	80
S.....	15	22	60	19	22	44	26	58	84
4. N.....	14	0	22	7	12	36	12	56	74
S.....	11	0	4	7	20	30	11	38	60
5. N.....	13	0	18	7	10	40	13	38	50
S.....	4	0	15	8	26	42	8	52	72
Averages.....	11.5	18.2	37.9	11.5	29.5	48.7	15.5	61.8	74.6

The following figures are derived from averages of animals

mgm. HCl.....	15.91	26.66	42.19
mgm. HCl/cc.....	1.38	2.32	2.72
Parietal secretion...	2.65	4.44	7.03
Nonparietal secretion.....	8.85	7.06	8.47

N = normal; S = saline.

phosphate intramuscularly and further specimens representing the secretory response over 10 minute periods were collected.

Two of the animals, nos. 2 and 3, received the washings from all five donor animals at different times, while the other three were used to test the material from one or more loops. In all, 16 tests were made in the five recipient animals.

The jejunal washings were found to be of pH 6.1 to 6.5 and to contain practically no titratable buffer. Thus, the pH was altered by 3 points when one drop of N/10 HCl or N/10 NaOH was added to 5 cc. of the washings.

As further controls, each of the five dogs used as recipients in the experiments were subjected to gastric analyses exactly comparable to those used to test the jejunal washings, except that saline alone was instilled.

RESULTS. *Effect on acidity of gastric juice.* While the specimen removed at the end of the 10 minute period during which the jejunal washings remained in the stomach always showed a marked reduction in the acidity, it seemed possible that the factor of dilution with an indeterminate portion of the 25 cc.

TABLE 2
Effect of washings of isolated jejunal loops on gastric secretion
Experimental series

GASTRIC ANALYSES					TIME AFTER HISTAMINE					
Animal		Fasting specimen			0-10 minutes			10-20 minutes		
Recipient no.	Donor no.	Vol.	Free acid	Total acid	Vol.	Free acid	Total acid	Vol.	Free acid	Total acid
		cc.			cc.			cc.		
1	6	5	20	50	7	12	22	8	92	110
	7	15	50	66	3	10	20	18	36	56
2	6	6	56	68	5	10	20	7	38	56
	8	9	30	58	24	5	16	16	10	26
	7	2	0	10	5	8	28	21	52	66
	9	16	70	88	24	14	30	33	66	88
	10	8	54	72	14	12	26	19	48	74
3	8	32	32	54	40	8	28	18	24	52
	7	33	20	44	5.5	0	20	14	30	54
	10	13	50	70	8	6	22	26	42	62
	9	6	16	40	7	4	34	12	22	54
	6	8	48	80	17	6	28	10	10	36
4	7	5	0	4	7	0	8	10	44	70
	6	11	0	44	4	0	10	14	20	10
5	6	4	0	5	4	0	7	7	38	58
	7	7	5	25	11	10	30	13	30	60
Averages.....		11.2	28.1	48.6	11.6	6.5	21.8	15.3	37.6	52.5

The following figures are derived from averages of above animals

mgm. HCl.....	19.86	9.23	29.32
mgm. HCl/cc.....	1.77	0.79	1.92
Parietal secretion...	3.31	1.54	4.89
Nonparietal secretion.....	7.89	10.06	10.41

of material instilled might have influenced the result, and for comparison with the control series this specimen was therefore discarded.

The comparative results in the experimental and control series appear in the accompanying table. From these figures it is apparent that while the injection of 1 mgm. of histamine phosphate is followed by a considerable rise in both the free and total acidities in the control series, there is a definite fall in both

of these values when the injection follows the instillation into the stomach of washings of isolated jejunal loops. By the end of 20 minutes the effect of the instillation has worn off to some extent.

The changes noted in the free and total acidities are reflected in the amounts of HCl secreted and in the milligrams of acid per cubic centimeter of gastric juice, the concentration being affected to a greater extent than the volume. It is interesting therefore to calculate the secretion of the parietal cells according to the method used by Gray, Bradley and Ivy (5). This is based on the statements of Lui, Yuan and Lim (6) and of Hollander (7) that the secretion of the oxyntic cells contains approximately 6 mgm. of HCl per cubic centimeter. Thus, by dividing the total milligrams of acid secreted by 6, one obtains a figure representing the volume of "parietal secretion" and by subtracting this from the total volume, an index of the mucous and pyloric or the diluting secretion. While these figures cannot be considered as more than estimates, they do provide a rough gauge of the activity of these cells.

Reference to these figures suggests that the depression in the acidity of the gastric juice is the result of a corresponding diminution in the activity of the oxyntic cells.

The depression of the normal reaction of the gastric analysis to histamine in the experimental series only lends support to the idea that this is due to a specific factor which can be obtained by washing out the lumen of the isolated jejunum with normal saline.

SUMMARY

When 25 cc. of saline washings of isolated jejunal loops are instilled into the stomach of normal dogs and withdrawn after 10 minutes there follows a marked depression in the normal response of the gastric acidity to histamine which is maintained for at least 20 minutes, suggesting a substance within the jejunal lumen which is responsible for the effect.

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STUDIES ON THE EFFECT OF ANOXIC ANOXIA ON THE CENTRAL NERVOUS SYSTEM

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Although the effect of anoxia on the electroencephalogram (EEG) of the human is well established (Davis) no observations seem to have been reported in which in unanesthetized animals the effect of anoxia over a wide range of oxygen tensions has been investigated. Such a study appears to be desirable as a means of determining the relation between the severity of anoxia, the type of cortical potential and the general behavior of the animal. In the present study several modifying factors have also been investigated such as the rôle of the rate of ascent as well as drugs and dietary factors.

METHODS. The experiments were performed on more than 100 rats in which the EEG and, in some experiments, the pulse rates were recorded with an Offner ink crystallograph. The rats were placed in glass jars of 1 gallon capacity and the barometric pressure was lowered according to table 1 by means of a Cenco vacuum pump. For further details compare Kessler and Gellhorn.

RESULTS. I. *The effect of "moderate" anoxia on the EEG.* Three series of experiments (cf. A., B., E. in table 1) were performed in which the effect of lowering the barometric pressure to 190 mm. Hg was studied on the EEG. Figures 1 and 2 illustrate the typical results obtained under these conditions on 38 rats. Figure 1 shows that shortly after the level of 190 mm. Hg has been reached the electrical potentials disappear almost completely. At this time the animal appears to be unconscious, does not react to any stimuli and is unable to right itself. This latter observation seems to indicate not only that cortical activity is practically eliminated as seen by the EEG record but also that the functions of the pons are likewise greatly depressed. This period of temporary silence occurs, as table 2 indicates, in 76 per cent of all experiments. In spite of the fact that the rat remains exposed to this lowered oxygen tension, there is a gradual resumption of electrical activity as illustrated in figure 1. Alpha potentials reappear and later periods of very large potentials of a frequency of 8 to 10 per second are observed. These spindles show an amplitude of 100 μ v or more and continue to be present at this barometric pressure until the end of the experiment (30 min.). Although spindles occur in practically all experiments of this type, their onset is somewhat variable. They appear earlier in those cases in which the sudden lowering of the barometric pressure does not lead to a period of complete cortical silence.

Figure 2 shows a record which is representative of a minority of experiments performed under the same conditions. In these cases no period of complete

¹ Aided by a grant from the Josiah Macy, Jr. Foundation.

cortical silence is observed but instead large delta waves appear. They decrease gradually in amplitude and then records are obtained in which spindles of a frequency of 8 to 10 alternate with small potentials similar to those seen at normal oxygen tension (fig. 2). Both types of observations have in common the fact that the rapid ascent leads at first to a more severe depression of cortical activity than is observed later after some adjustment reactions have taken place. When rapid, small potentials alternate with spindles, the rats are still markedly depressed but the righting reflex has been restored.

TABLE 1
Procedure of decreasing barometric pressure

TIME	PRESSURE				
	A	B	C	D	E
<i>minutes</i>	<i>mm. Hg</i>	<i>mm. Hg</i>	<i>mm. Hg</i>	<i>mm. Hg</i>	<i>mm. Hg</i>
1	580	580	580	580	580
2	480	480	480	480	550
3	380	380	380	380	500
4	280	280	280	280	450
5	190	190	190	190	400
6					350
6-10				190	
6-15		190			
6-36	190		190		
7-10					300
11-15					280
11-16				160	
16-20					260
16-46		160			
17-22				140	
21-25					240
23-28				120	
26-30					220
29-39				110	
31-60					190
37-67			140		

In order to determine the cause of the disappearance of the silent period or of the period of large delta waves which occur early during the anoxia, blood pressure records would be of greatest importance. Unfortunately, such records could not be obtained for the present set of experiments but the pulse rate was recorded (table 2). The records showed that the pulse rate was regular at that time and not essentially different from that of the control values obtained at 750 mm. Hg or from the rate observed after recovery from the silent period had been achieved.

In spite of it, it appears to us rather probable, unless blood pressure experiments would prove the contrary, that the restoration of cortical potentials is due to an improvement in the oxygenation of the brain rather than to an adaptation of the cortical cells to a low oxygen tension.

If the barometric pressure is lowered from 190 mm. Hg to 160 mm. Hg after rats have been exposed to the former for 10 minutes (cf. column B in table 1) it is found that spindles also occur at 160 mm. Hg. They are likewise observed in experiments in which the level of 190 mm. Hg is reached very gradually (cf. column E of table 1). Under these conditions, spindles are not preceded by either a period of complete silence or delta waves (table 2). If the barometric

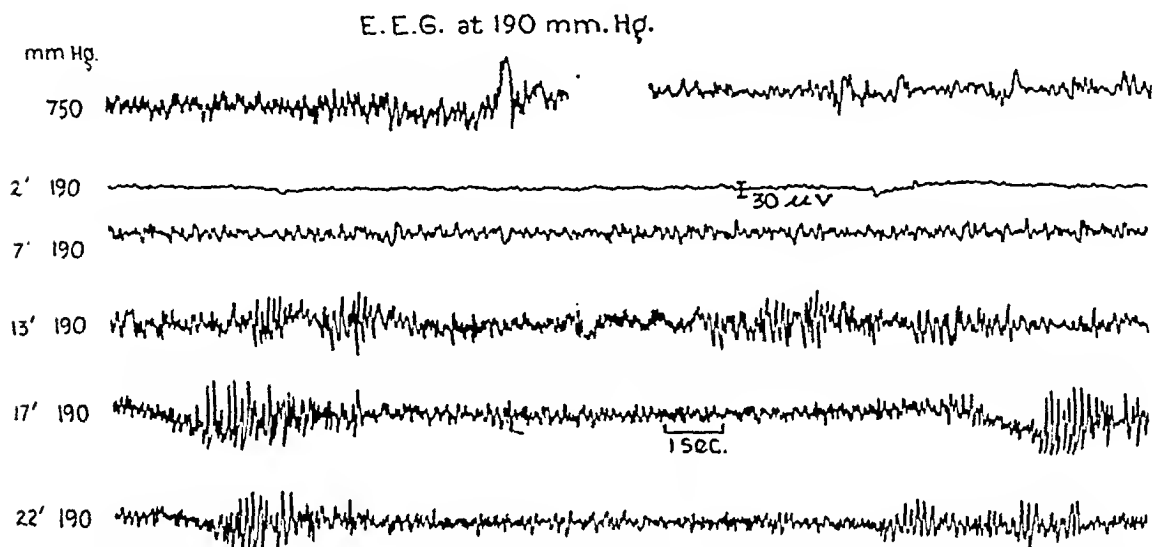


Fig. 1. The effect of reduced barometric pressure on the electroencephalogram of the unanesthetized rat. The pressure was reduced from 750 mm. Hg to 190 mm. Hg in five minutes (cf. table 1A).

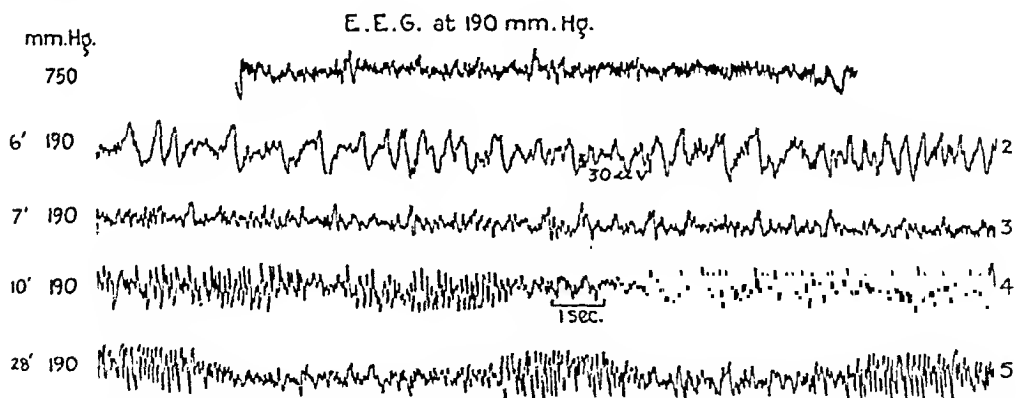


Fig. 2. The effect of reduced barometric pressure on the electroencephalogram. Procedure as in figure 1.

pressure is lowered more rapidly to levels below 190, spindles do not occur at 190 or 160 mm. Hg. The experiments suggest that spindles are characteristic of a certain range of oxygen tensions since they were almost never observed at a barometric pressure above 400 mm. Hg and were likewise absent at pressures of 140 mm. Hg or below. Another condition for their appearance is a time factor inasmuch as very rapid ascent through the indicated range does not lead to spindle formation.

II. *The effect of "severe" anoxia on the EEG.*² Two procedures (cf. C and D in table 1) were used in order to lower the barometric pressures below 160 mm. Hg. In procedure C a level of 140 mm. was reached in 36 minutes. Figure 3 shows that the lowering of the barometric pressure to 140 mm. Hg abolished the spindles seen at 200 mm. Hg. All potentials gradually decline in amplitude and

TABLE 2
Effect of decreased oxygen tension on the electroencephalogram

NO. OF RATS	PERIOD OF CORTICAL SILENCE			SPINDLES		REMARKS
	Per cent	Onset	Duration	Per cent	Onset	
38	76	Av. 1.6' (1'-5')	Av. 4.4' (1'-7')	95	Av. 16.3'* (7'-23')	Ascent to 190 mm. Hg in 5 min. (Method A)
12	0		0	92.5	Av. 21.2' (4'-37')**	Ascent to 190 mm. Hg in 30 min. (Method E)

* Time in minutes from the beginning of ascent.

** One rat showed spindles 4 minutes after beginning of ascent at which time the pressure was 450 mm. Hg; the rest developed spindles between 280 and 200 mm. Hg. Cf. table 1

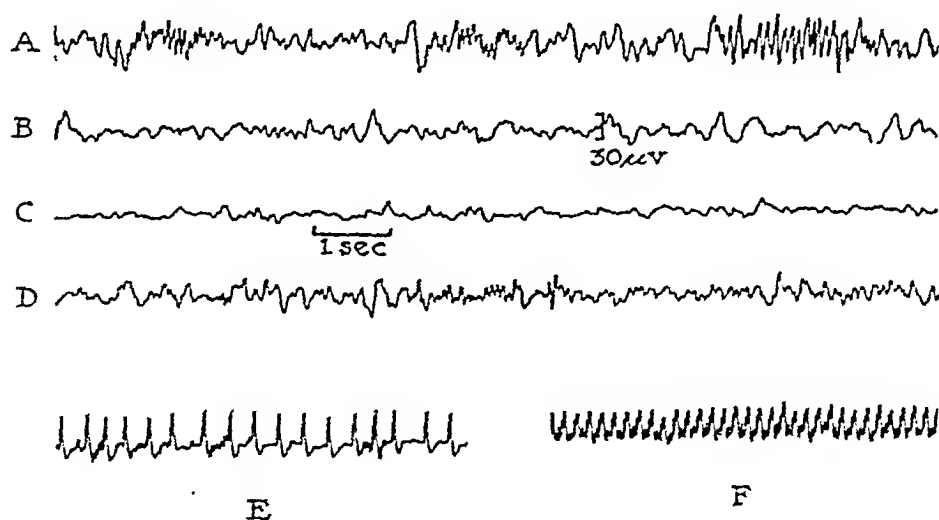


Fig. 3. The effect of lowered barometric pressure on the electroencephalogram. Procedure C of table 1. A, 13 min. at 200 mm. Hg; B, 2 min. at 140 mm. Hg; C, 12 min. at 140 mm. Hg; gasping; D, 10 min. after readmission of air; E, pulse rate taken during record C; F, pulse rate taken during record D.

become slower. Gasping occurs in most instances within 30 minutes (average 20 min.). At that time only few and slow potentials are seen. If the barometric pressure is not immediately increased, the animal dies. The occurrence of slow gasps indicates that under these conditions not only cortex and pons are

² The term "severe" anoxia has been used in this paper to denote the involvement of the medullary centers.

greatly depressed, since cortical potentials are practically absent and righting reflexes are abolished, but that the medulla oblongata is likewise affected (Lumsden). At the same time the recording of the pulse rate indicates gross irregularities and slowing (cf. record E in fig. 3). Readmission of air restores cortical potential, normal behavior, and regularity of the pulse (cf. records D and F in fig. 3).

In 11 animals the barometric pressure was lowered rather rapidly to 110 mm. Hg (procedure D of table 1). Of the 11 animals, 10 gasped at a pressure varying between 140 and 110 mm. Hg. Here again gasping was accompanied by a slow irregular pulse, absence of cortical potentials and loss of righting reflexes.

III. *Factors modifying the effect of lowered barometric pressure on the EEG.* In fifteen experiments, the effect of benzedrine (5 mgm./kgm., intraperitoneally) was studied on the EEG of rats subjected to procedure A. It was found that a

TABLE 3
Effect of decreased barometric pressure (190 mm. Hg.—Method A) on pulse rate

RAT	PULSE RATE PER MINUTE		
	Control	190 mm. Hg	
		Cortical silence onset	Recovery onset
1	420	400	388
2	390	412	410
3	374	378	388
4	366	313	340
5	532	456	500
6	480	420	434
7	400	448	450
8	458	384	382
Average.....	428	401	412
Per cent.....	100	94	96

period of silence was observed in 9 out of 15 experiments (60 per cent), a figure which is not essentially different from that seen in uninjected controls (76 per cent). However, spindles did not occur after the recovery from the period of silence in any of these animals (fig. 4) whereas they were found in 95 per cent of the control animals. At the same time it was observed that the rats, instead of being depressed as uninjected controls were at 190 mm. Hg. during the time when spindles occurred, appeared entirely normal: they moved about in the low pressure chamber and reacted to external stimuli. If benzedrine injected rats were subjected to procedure B in which the pressure was lowered to 160 mm. Hg, the EEG failed again to show spindles which regularly occurred in the corresponding control experiments.

It is important to note that this fundamental difference in the reactivity of the cortex existing between "benzedrine" and control rats is limited to the range of oxygen tensions of 160 to 200 mm. Hg since entirely different results were ob-

tained under conditions of "severe" anoxia. Benzedrine rats which were subjected to procedure D showed in all nine experiments that gasping occurred between 140 mm. Hg and 110 mm. Hg at times similar to those observed under control conditions; moreover, experimental and control rats showed a similar behavior and similar changes in EEG. These experiments suggest that observations made on animals subjected to a moderate degree of anoxia may not permit

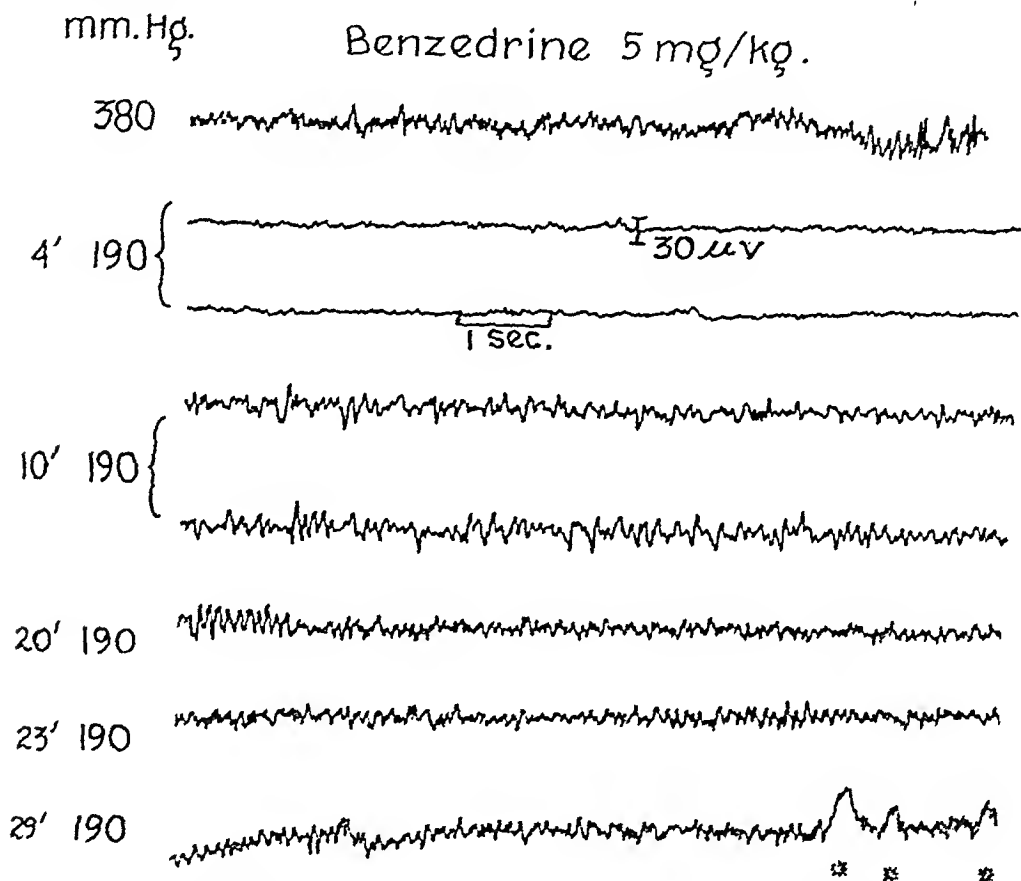


Fig. 4. The effect of benzedrine (5 mgm./kgm.) on the electroencephalogram of the unanesthetized rat under conditions of lowered barometric pressure. Asterisks indicate movements.

one to make any prediction with regard to their behavior at extreme degrees of anoxia and vice versa.

In a number of investigations the resistance of animals to anoxia under various conditions has been tested by determining their survival time under conditions of extreme anoxia. Thus Campbell found that carrot-fed rats show an increased resistance to anoxia. We were able to confirm this finding on fifteen rats since only six rats (40 per cent) gasped within 10 minutes at 100 mm. Hg, whereas in rats fed dog chow diet, ten of eleven rats (91 per cent) gasped within this period. On the other hand, it was shown that this increased resistance displayed by the carrot-fed rats under conditions of "severe" anoxia did not appear when the

animals were subjected to "moderate" anoxia (procedure A of table 1). Under these conditions behavior and EEG were similar and apparently independent of the diet.

DISCUSSION. With respect to the reversible period of electrical silence of the cortex which occurs, after rapid ascent, at a barometric pressure of about 190 mm. Hg it was found that in spite of continued anoxia, the brain potentials gradually recover to the "spindle" type. The absence of changes in pulse rate when recovery from this period of temporary cortical silence occurs suggests that systemic circulatory changes are not involved in the recovery. It is, however, not improbable that an improved oxygenation of the cortex is the cause of this recovery, since dilatation of cerebral vessels may gradually increase in anoxia particularly if acid metabolites are accumulated at the same time.

The period of electrical silence of the cortex observed at a barometric pressure of 190 mm. Hg is fundamentally different from that observed at pressures of 140 mm. and below since the latter ends invariably in death unless the barometric pressure is quickly raised. The reversible form of electrical silence (at 190 mm. Hg) occurs under conditions indicating an anoxic involvement of cortex and brain stem down to the pontine level (righting reflexes). The irreversible form of cortical silence (at 140 mm. Hg) is accompanied by an involvement of the whole brain including the medulla oblongata as indicated by gasping, slowing and irregularities of pulse rate suggesting vagal discharges. If the barometric pressure is restored rapidly, the animals may completely recover. The observations made at 190 mm. Hg on one hand and at 110 to 140 mm. Hg on the other hand give renewed evidence for the well established fact that anoxia affects more easily cortex and brain stem than medullary centers (cf. Gellhorn, 1943, p. 166). They do not support Windle's claim that anoxia results in fortuitous damage to various parts of the central nervous system.

The spindles observed at 190 mm. Hg are similar to those reported by Sugar and Gerard following recovery from complete anemia of the brain and interpreted by them as an indication of increased excitability of the cortex. Since their experiments were carried out in narcotized animals, no comparison with the general behavior could be made. It is, therefore, of interest to emphasize that our observations clearly showed a marked depression of the animals during the period in which trains of spindles prevailed. Whereas the animals showed no spontaneous movements at 190 mm. Hg and appeared to have also a diminished tone of the muscles, they assumed normal activity and behavior as soon as the pressure was raised to a level at which these spindles disappeared.

Our experiments have confirmed Campbell's findings but have shown that in spite of the greater resistance of carrot fed animals to lethal tensions of oxygen, they appear not to be more resistant to milder degrees of anoxia inasmuch as they show the same cortical and clinical changes as do control rats. Similarly, it has been found that benzedrine which decidedly improves the performance of rats at 190 mm. Hg does not in any way alter their resistance to lethal tensions of oxygen. It must be concluded that experiments done under conditions using

the time of survival under severe anoxia as the criterion of anti-anoxic properties of certain drugs or procedures are more of pharmacological than physiological interest.

SUMMARY

Studies on the effect of lowered barometric pressure on unanesthetized rats have shown that when the ascent is carried out rapidly a period of temporary silence of the cortex occurs at a level of 190 mm. Hg from which the animals recover spontaneously in spite of continued lowered barometric pressure. During the period of cortical silence righting reflexes are absent. Recovery is accompanied by the appearance of large spindles of a frequency of eight to ten per second. Righting reflexes reappear during this period but the animals remain depressed. Rats injected with benzedrine show similar changes in EEG as far as the period of cortical silence is concerned, but they show during the subsequent recovery a normal EEG without spindles and without any other signs of depression of activity. If the pressure is gradually lowered to 190 mm. Hg, the periods of temporary silence may be absent, but spindles accompanied by a general depression of the animals are found. The occurrence of spindles is confined mostly to barometric pressures varying between 280 mm. Hg and 160 mm. Hg. If the pressure is lowered to 110 to 140 mm. Hg, a period of electrical silence occurs from which no recovery at that barometric pressure is possible. As the gasping and slowing of pulse show, the medulla is involved under these conditions.

Benzedrine which increases the resistance of rats to anoxia as indicated by the EEG at moderate degrees of anoxia does not increase the resistance or the survival time of rats under conditions of extreme anoxia. Conversely, rats fed with carrots although showing an increased survival time under lethal conditions (Campbell) do not show any improvement in performance or in EEG under conditions of more moderate anoxia.

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THE EFFECT OF ANOXIC AND ANEMIC ANOXIA ON THE LEUCOCYTE COUNT

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In a previous investigation it was shown by the present authors that chemically (metrazol) and electrically induced convulsions call forth a neutrophilic leucocytosis in normal animals. However, no changes in the leucocyte count occurred in adreno-demedullated rats subjected to the same measures. These data indicate that leucocytosis may be the result of an excitation of centers of the sympathetico-adrenal system leading to a discharge of adrenalin which by direct action on the bone marrow results in an increased discharge of neutrophilic leucocytes. In addition to this mechanism other mechanisms are involved also which appear to be independent of the discharge of adrenalin. It was found that the injection of typhoid-paratyphoid vaccine led to a neutrophilic leucocytosis which was not confined to the normal animals but was also present in adreno-demedullated rats to a similar degree (Clare, Cress and Gellhorn, 1943).

Extensive studies (cf. Gellhorn, 1943, for literature) have shown that anoxia causes an excitation of the sympathetico-adrenal system. It is likewise known that anoxia, at least in its earlier stages, is accompanied by a leucocytosis later followed by leucopenia (Meyer, Seevers and Beatty, 1935; Ruppner, 1920). The question, therefore, arises as to whether anoxia causes leucocytosis by means of sympathetico-adrenal discharges. Since it is known that not only anoxic anoxia but also anemic anoxia (Nasmith and Graham, 1906) call forth leucocytosis as well as excitation of the sympathetico-adrenal system, it was decided to investigate the dependence of leucocytosis on the sympathetico-adrenal system under these conditions.

METHOD. The experiments were performed on 52 rats averaging 250 grams in weight. Half of the animals were adreno-demedullated. Blood samples were obtained from the tail after amputation of 1 or 2 mm. and discarding of 2 or 3 drops. Control experiments reported in our previous work showed only insignificant changes in leucocyte count taken at two hour intervals over 24 hrs. Operated animals were used not earlier than 3 to 4 weeks after operation. No food was given during the periods of anoxia.

Anoxic anoxia was established by subjecting the animals to a barometric pressure of 400 mm. Hg in a low pressure chamber for 12 hrs. on each of four successive days. Before and after the period of anoxia red and white counts were taken. The effect of anemic anoxia was studied in two ways: 1st, by subjecting rats to carbon monoxide poisoning, and 2nd, by hemorrhage. Carbon monoxide was found to be effective when injected intraperitoneally (1 cc. per 20 grams' body weight). Blood counts were taken before, and 6 and 12 hrs. after the injection.

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In order to study the effect of hemorrhage, about $\frac{1}{3}$ of the total blood was removed from the tail in 30 minutes. It was assumed that the rat has 6.7 cc. of blood per 100 gram of body weight (Griffith and Ferris, 1942). Blood counts were taken before bleeding and 13, 17 and 24 hrs. after bleeding. A differential count was carried out on 10 normal rats subjected to anoxic anoxia and CO poisoning.

RESULTS. The effect of anoxic anoxia on 5 of the 10 animals which were investigated is shown in table 2. It is clearly evident that anoxic anoxia causes a decided increase in leucocytes in normal but not in adreno-demedullated rats. The leucocytosis occurs in most instances at the end of the first period of anoxia; only in two out of ten animals was the leucocytosis at its maximum after the second anoxic period. Thereafter, there was a gradual decline in leucocyte count so that at the end of the fourth anoxic period the count was on the average only slightly above the control levels. The effect is not due to hemoconcentration as evidenced by the fact that the changes in erythrocytes during these experiments

TABLE 1
Effect of various forms of anoxia on the white cell count

NO. OF RATS	AVERAGE WHITE COUNT IN mm^3 (CONTROL)	ANOXIA	AVERAGE WHITE COUNT IN mm^3 (AFTER ANOXIA)	INCREASE IN % OF CONTROL	PERCENTAGE		PERCENTAGE		PERCENTAGE INCREASE IN NEUTROPHILS
					Neutrophils before anoxia	Lymphocytes before anoxia	Neutrophils after anoxia	Lymphocytes after anoxia	
5	21080	CO 1 cc./20 grams weight intraperitoneally	28860	37	30	70	62.5	37.5	108
5	14550	16 hrs. at 300 mm. Hg	18000	24	26.8	73.2	43.2	56.8	61

were only very slight. After the end of the first anoxic period the leucocytes in 10 rats had increased from 18,000 to 30,000 per mm^3 whereas the red counts increased from 9.4 to 9.7 millions per mm^3 .

None of the adreno-demedullated rats showed a leucocytosis. In two adreno-demedullated rats (nos. 5 and 8; the record of the latter is not shown in this paper) there was a decided fall in leucocytes but in most observations no significant effects were observed. Whereas the maximal increase in white count of 10 normal rats averaged 82 per cent, it amounted to 4.7 per cent only following anoxia in 10 adreno-demedullated rats.

The influence of severe hemorrhage on the blood count on normal and adreno-demedullated rats is illustrated by table 3. Here again it is shown that leucocytosis is confined to the normal group and appears at its height 8 to 12 hrs. after bleeding. Hereafter, the white count gradually returns to control levels. For the interpretation of these findings, the curve illustrating the red count is important. It shows that four hours following the bleeding, the red count is reduced to approximately 50 per cent of the original value and remains at this

TABLE 2

Effect of low barometric pressure (12 hrs. at 400 mm. Hg) on the leucocyte count

HOURS	PROCEDURES	A. NORMAL RATS										B. ADRENODEMEDULLATED RATS									
		#1		#2		#3		#4		#5		#1		#2		#3		#4		#5	
		E*	L†	E	L	E	L	E	L	E	L	E	L	E	L	E	L	E	L	E	L
0	Control Counts	9.6	15.6	9.0	13.5	9.1	15.0	9.3	20.0	8.9	18.0	9.7	28.0	8.9	19.0	8.5	19.5	9.3	18.0	8.0	24.0
			16.0		14.0		14.5		21.0		17.8		28.5		19.5		19.0		19.0		24.4
1-13	Anoxia																				
13	Counts	9.5	16.0	9.4	34.0	9.2	27.0	9.5	32.5	9.1	39.2	10.4	30.5	9.4	19.4		18.0	9.6	18.6	7.8	22.0
13-25	Rest																				
25-37	Anoxia																				
37	Counts	10.4	23.5	9.6	26.0	10.0	25.0	9.3	21.0	10.0	26.0	10.3	31.0	9.1	18.5		19.5	9.5	18.3	6.8	25.0
37-49	Rest																				
49-61	Anoxia																				
61	Counts	10.5	21.0	9.5	24.0	10.1	21.0	9.7	22.0	10.1	23.0	9.8	28.0	11.0	23.0		19.5	9.4	20.0	8.0	17.0
61-73	Rest																				
73-85	Anoxia																				
85	Counts	10.4	19.0	9.5	21.0	9.8	18.0	9.5	20.5	10.0	20.0	9.0	29.0	10.0	16.0		19.0	9.6	18.5	8.2	19.2
L maximal increase (%).....		48.7		146.1		82.5		58.5		118.9		9.5		19.2		1.0		8.1		3.3	

Average maximal increase of L in 10 normal rats = 81.7%. Average maximal increase of L in 10 adrenodemodulated rats = 4.8%.

* E = Erythrocytes in millions per cu. mm.

† L = Leucocytes in thousands per cu. mm.

TABLE 3

Bleeding experiments

RAT	WGT.	CON- TROL E	CON- TROL I L	CON- TROL II L	CC. BLOOD RE- MOVED	LEUCOCYTES						% INCR. IN L	ERYTHROCYTES						
						3 hrs.	7 hrs.	11 hrs.	13 hrs.	17 hrs.	24 hrs.		3 hrs.	7 hrs.	11 hrs.	13 hrs.	17 hrs.	24 hrs.	
						After bleeding							After bleeding						
Normal rats																			
	<i>gms.</i>																		
1	210	9.0	18.5	16.0	4.0	15.8	21.2	31.3	25.7	23.0	19.2	81.0	5.5	3.9	4.2	4.0	4.5	4.8	
2	200	9.0	18.5	17.0	4.0	19.0	13.7	24.8	22.4	16.5	20.0	39.2	5.4	3.8	4.2	4.6	4.5	5.0	
3	220	8.5	20.0	21.0	4.5	16.0	40.0	27.0	32.5	19.5	29.5	99.1	5.1	5.2	5.0	5.4	5.5	5.2	
4	230	10.0	20.0	22.0	4.5	26.0	35.0	29.8	25.0	23.5	24.0	66.6	5.1	4.1	4.0	4.2	4.5	4.4	
5	240	8.9	20.0	19.0	5.5	16.3	30.0	35.0	27.5	24.0	19.2	79.5	5.0	5.7	5.2			5.0	
6	280	9.0	22.3	22.0	5.5	22.5	39.0	28.0				75.1	5.8	5.5	5.9				
Average												73.4							
Adreno-demedullated rats																			
1	225	9.8	30.0	29.0	4.5	20.4	19.4	20.1	20.6	22.0	22.6	-34.1	5.8					6.0	
2	275	9.7	19.0	18.9	5.5	17.0	20.0	17.3	18.0	20.0	21.2	11.6	5.6	5.2	5.3	5.0	5.2	5.3	
3	295	9.6	20.3	19.0	5.5	17.5	13.0	18.0	17.8	16.8	16.0	-33.6	5.9					4.7	
4	235	8.45	27.0	26.0	5.0	20.0	24.0	25.0				-24.6	6.2	5.0	6.0				
5	240	9.8	28.0	27.0	5.0	15.0	22.0	24.0				-20.0	6.0	5.2	5.9				
6	165	8.9	22.0	23.0	5.0	24.0	22.5	23.0				6.7	5.6	4.9	5.0				
Average												-15.7							

level for the next 24 hours indicating that the blood volume has increased considerably. In spite of this dilution, the increase in leucocytes averages 72 per cent in six normal rats. The actual production and release of leucocytes into the circulatory system must have been considerably greater than is indicated by this figure.

The adreno-demedullated rats show in 4 out of 6 animals a decrease in white count after bleeding which roughly parallels the decrease in red count and may

TABLE 4
Effect of CO on the leucocyte count*

RAT	CONTROL E	CONTROL I L	CONTROL II L	LEUCOCYTES			% INCR. L	E 6 HRS. AFTER CO	E 12 HRS. AFTER CO
				2 hrs. after CO	6 hrs. after CO	12 hrs. after CO			
Normal rats									
1	8.8	21.0	20.0	17.5	28.2	27.0	37.5	8.0	8.0
2	9.0	20.0	20.0	15.0	27.8	24.5	39.0	8.5	8.6
3	9.0	30.0	29.7	21.8	40.0	37.0	33.3	9.1	9.0
4	9.5	17.5	16.0		32.0	28.0	91.6		
5	9.0	20.7	20.0		32.0	25.0	56.8		
6		21.0	18.5		30.0	22.0	51.5		
7		19.8	16.0		31.0	24.0	73.1		
8		23.2	20.0		21.0	36.0	66.6		
9		17.4	17.0		15.2	33.5	94.7		
10	9.4	25.0	23.0		36.5	32.0	52.1	9.0	
Average.....							54.4		
Adrenodemedullated rats									
1		23.6	22.0	27.6	27.4	25.0	20.1		
2		32.2	31.0	31.0	32.0	29.0	1.3		
3	8.7	28.0	27.0	27.0	26.0	26.5	-5.0	8.9	9.0
4	8.9	30.0	28.0		31.0	31.5	8.6	9.0	8.8
5	8.9	20.0	19.0		23.0	22.0	17.9	8.9	
6	9.3	19.0	18.5		21.0	21.0	11.7	9.0	
7	9.8	22.4	22.3		23.0	21.0	2.7	9.2	
8	8.5	28.0	27.0		27.5	27.0	-1.9	8.4	
9	9.0	29.0	28.0		28.0	29.0	1.8	9.3	
10	9.4	25.6	24.0		26.0	24.0	4.9	9.5	
Average.....							7.6		

* 1 cc. CO per 15 to 20 grams weight, intraperitoneally.

be accounted for by the dilution of the blood. In two rats the leucocytes show an insignificant rise (7 and 12 per cent respectively).

Normal rats under carbon monoxide poisoning show an increase in the number of leucocytes which is maximal after 7 or 13 hrs. (table 4). It amounts to 60 per cent on the average whereas the average change in the demedullated group is 6 per cent. After 24 or 48 hrs., the control value was obtained again in the unoperated group. These changes in leucocyte count were not accompanied

by any significant changes in the red blood count. Table 1 shows the leucocytosis which occurs in another group of normal rats under conditions of anoxic and anemic anoxia. Although the leucocytosis was of a lesser degree than in the main group of experiments described above, its neutrophilic character is unmistakable.

DISCUSSION. The experiments show conclusively that anemic and anoxic anoxia lead to a leucocytosis in normal but not in adreno-demedullated rats. The data suggest that the liberation of adrenalin in the normal group is the cause of the leucocytosis. This assumption is supported by the fact that anemic as well as anoxic anoxia leads to the liberation of adrenalin. That the results are not due to changes in blood volume is indicated by the simultaneous determination of the red count which does not show any significant changes in anoxic anoxia and in carbon monoxide poisoning in the present investigation. In the group showing anemic anoxia as a result of hemorrhage, there was a fall in red blood count indicating a marked dilution of the blood but this fall was similar in normal and adreno-demedullated rats. Nevertheless, only the normal group showed leucocytosis.

The experiments agree with our earlier studies in which metrazol and electroshock caused a leucocytosis which under these conditions was likewise confined to the normal and was absent in the adreno-demedullated group. Moreover, in both groups of experiments, it was found that the leucocytosis was neutrophilic only. Both investigations support the assumption that sympathetico-adrenal discharges are a potent factor in the elicitation of a neutrophilic leucocytosis. However, chemical substances may also have a direct peripheral effect on the bone marrow as shown by the fact that after injection of typhoid vaccine, leucocytosis appears in both normal and adreno-demedullated animals.

SUMMARY

The effect of anoxic and anemic anoxia is studied on the blood picture of normal and adreno-demedullated rats. It is found that anoxic anoxia induced by exposure of the animals to a barometric pressure of 400 mm. Hg for 12 hrs. on four successive days causes a leucocytosis after the first or second period of anoxia in normal but not in adreno-demedullated rats. Similarly, it is observed that carbon monoxide poisoning and hemorrhage are followed by a leucocytosis confined to the normal group. The leucocytosis is in all cases of a neutrophilic character. The experiments indicate that the leucocytosis in anoxic and anemic anoxia is due to a discharge of adrenalin following the excitation of the sympathetico-adrenal system. It is assumed that the liberated adrenalin acts directly on the bone marrow.

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THE EFFECTS OF POLYCYTHEMIA AND OF A CARROT DIET ON RESISTANCE TO ANOXIA

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Interest in measures designed to increase resistance to anoxia is understandably acute at the present time. Since one of the most constant physiologic reactions to anoxia is the increase in the number of red cells the possibility that an artificial polycythemia induced by blood transfusions might be beneficial to high altitude fliers merits investigation. Considering the ease of the operation and the long life of the red cell, such a method, if effective, might be quite practical. Authorities differ, however, on the importance of polycythemia as a factor in acclimatization as is evident on reading the chapter "Acclimatization" in *Anoxia, Its Effect on the Body*, by Van Liere, (1). Experimentally, Campbell (2, 3, 4) has studied the effects of an increased hemoglobin percentage on tissue oxygen tension, using the method of injected nitrogen bubbles, but we could find no reports concerning its effect upon actual resistance to anoxia.

In attacking this problem we were impressed by the great individual variation in the survival time of rats under conditions of acute anoxia. It also seemed to us that since the survival of high altitude personnel depended in large part upon the reception of sensory stimuli and the ability to respond to them, a test based upon the loss of a reflex response under anoxic conditions might be more appropriate than length of survival. Such a method was therefore developed and applied to the polycythemia problem.

Finally, the confirmation by Nelson, Goetzel, Robins and Ivy (5) of the original report of Campbell (6) that a ten day carrot diet markedly increased the resistance of rats to acute anoxia, raised the question whether such increased resistance would also be shown by our test.

EXPERIMENTAL. Albino rats of the University of Denver strain were used in these studies. The method employed was as follows. An electric bicycle horn was placed in the bottom of a vacuum desiccator, the leads sealed through the stopper and the horn operated by means of an outside switch. A wire frame, on which the animal rested, was placed over the horn so that he would not be affected by the mechanical vibration of the horn. Under these conditions, sounding the horn produced a uniform response, namely, a sharp, backward twitch of the ears, which we call the "Ear-batting Reflex," which is easily observed through the walls of the desiccator. Undue excitement is not produced, a rat lying quietly in the chamber will usually merely bat his ears when the horn is sounded.

Exposure to low barometric pressures results in loss of this reflex, after varying lengths of time. (We believe that failure of the animal to respond is probably due to its failure to hear the signal rather than to muscular paralysis, at least the

ability to respond to electrical stimulation persists much longer.) Disappearance of the reflex, when tested at 30 second intervals is usually unequivocal, that is, the last response may have been quite strong but 30 seconds later it will have

TABLE 1

Control reflex times, in minutes, for 5 determinations, made at weekly intervals

RAT NO.	1ST	2ND	3RD	4TH	5TH	AVERAGE	σ
17	14	10	7	6	10	9.4	2.8
4	14	14	12	13	14	13.4	.8
11	13	15	24	18	14	16.8	4.0
3	32	35	31	34	32	32.8	1.5
16	24	40	30	48	33	35.0	8.2

TABLE 2

Effects of polycythemia on reflex time

RAT NO.	BEFORE TREATMENT			TREAT- MENT*	AFTER TREATMENT					
	RBC†	Reflex time‡	σ		1 week		2 weeks		3 weeks	
					RBC	Reflex time	RBC	Reflex time	RBC	Reflex time
				cc.						
1	7.85	9.8	1.6	2.25	10.20	12	10.05	15	8.00	14
2	8.55	16.6	1.5	2.25	9.85	20	9.60	19	9.15	16
3	8.20	32.8	1.5	2.25	9.95	25	10.15	15	7.65	30
4	7.90	13.4	.8	3.2	11.60	16	10.40	14	7.40	14
5	9.0	22.0	3.5	3.3	10.60	19	10.10	25	9.10	22
6	8.35	24.6	2.8	3.15	9.65	13	9.60	22	9.00	28
Av.....	8.31	19.9			10.31	17.5	9.98	18.3	8.38	20.7
7	9.00	11.2	3.2	6.2	14.20	16	11.40	16	8.75	9
8	8.00	15.0	1.7	6.25	12.65	12	11.70	15	9.50	13
9	8.10	15.0	3.6	6.0	13.80	20	12.80	14	10.30	18
10	9.10	13.0	1.3	6.5	14.80	10	11.55	9	10.20	11
11	8.40	16.8	4.0	6.25	12.90	12	12.75	6	8.55	9
12	8.75	17.2	2.4	6.25	11.75	19	9.60	21	9.15	16
Av.....	8.56	14.7			13.35	14.8	11.63	13.5	9.41	12.7

* Red cells suspended in saline solution. The amounts given represent the volume of whole blood from which red cells were obtained.

† Red blood count in millions.

‡ Average of 5 determinations at weekly intervals before transfusion. Reflex time means the number of minutes elapsing between the time an altitude of 40,000 feet was reached and the time at which the reflex disappeared.

disappeared completely. Sometimes an animal failing to respond to the usual blast will respond if the signal is prolonged or repeated, but after another 30 seconds no response whatever can be elicited. Once lost, the reflex does not reappear unless the pressure is raised, whereupon it reappears promptly. It

therefore seems reasonable to assume that loss of the reflex is due to anoxia and the length of its survival measures the animal's resistance to this condition.

As regards variability, raw data on 5 of the rats used in this study are presented in table 1. These 5 rats represent the extremes of consistency and inconsistency and also the extremes of time of reflex survival. All other rats fall between these extremes, as shown by the standard deviations and average reflex times listed in tables 2 and 3. It will be noted that the variations in a given rat, determined from time to time, are less than the variations between different rats, which suggests the advisability, in future work, of using animals selected for their uniformity.

All determinations were made at a pressure of 140 mm., corresponding to an altitude of 40,000 feet, the rate of ascent being 2,000 feet per minute. The desired barometric pressure was obtained by means of a vacuum pump, air being admitted through an adjustable capillary. The circulation through the chamber at 40,000 feet was 4 liters of outside air per minute. After reaching

TABLE 3
Effect of carrot diet on reflex time

RAT NO.	AV'G. REFLEX TIME (5 DETERMINATIONS)	σ	REFLEX TIME, 10TH DAY	REFLEX TIME, 11TH DAY
13	21.8	3.2	19	18
14	10.0	1.8	13	19
15	17.6	1.5	17	21
16	35.0	8.2	56	45
17	9.4	2.8	12	11
Av'g.....	18.8		23.4	22.8

40,000 feet the horn was sounded at 30 second intervals and the period elapsing between reaching that altitude and the disappearance of the reflex, read to the nearest minutes, recorded as the "Reflex time."

The normal reflex time of 12 rats was determined by five tests made at weekly intervals. Their blood count was determined, following which they were injected, via the jugular vein, with rat red cells suspended in saline in the amounts, expressed in equivalents of whole blood, shown in the table. Tests were again made at weekly intervals for three weeks and after each test a red count was made. The results are shown in table 2.

To study the effects of a carrot diet 5 rats whose reflex times had been established as described were placed on such a diet for 11 days. The weight loss was 8.7 per cent. Their reflex times were again determined on the 10th and 11th days. These results are shown in table 3.

DISCUSSION. It will be noted that two levels of polycythemia were produced. In the first group of six rats (table 2), which received red cells equivalent to a blood transfusion of 2.25 to 3.3 cc. each, the increase in red count was 24 per cent, while in the second group, receiving from 6.0 to 6.5 cc. the increase was 56

per cent. This was done to take into account the possibility that the advantage of a high red count might be overbalanced by the greater blood viscosity. At neither level, however, was there an increased resistance to anoxia. This agrees with the conclusion reached by Campbell (4) that an increased amount of hemoglobin at high altitudes is of no value.

As regards the carrot diet, the increase in resistance shown is slight and statistically insignificant. Although, using the percent of animals surviving acute anoxia as the criterion, Ivy's work (6) is conclusive in proving beneficial effects, this diet would appear to have little value under conditions where survival depended upon reflex responses, insofar, at least, as the response used in this study may be regarded as representative.

CONCLUSIONS

1. A method of studying anoxia in the rat, based on the loss of a reflex response to sound, is described.
2. Using this method, no increase in resistance to anoxia was produced either by a moderate (24 per cent) nor high (56 per cent) degree of polycythemia.
3. A 10 day diet of carrots exclusively was also ineffective.

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WATER AND FAT CONTENT OF ORBITAL TISSUES OF GUINEA PIGS WITH EXPERIMENTAL EXOPHTHALMOS PRODUCED BY EXTRACTS OF THE ANTERIOR PITUITARY GLAND¹

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When anterior pituitary gland extracts are injected into thyroidectomized guinea pigs, an exophthalmos and hypertrophy of the retrobulbar fat and extraocular muscles is produced (1, 7, 9). Study of histological sections of the orbital fat and muscles of such animals have shown that there is an increase in the quantity of interstitial water in these tissues. It has been impossible to determine, however, whether the lipid and the connective tissue constituents were increased in amount. For this reason quantitative determinations were made of the water, lipid (ether soluble) and connective tissue components of the orbital fat, and of the water content of the extraocular muscles of 87 exophthalmic and control female guinea pigs. The water content of peritoneal depot fat and of skeletal muscle of 70 of these animals was also determined.

MATERIALS. A marked exophthalmos was produced in 19 thyroidectomized guinea pigs by the injection of an extract of anterior pituitary glands. In addition, 17 normal animals and 26 thyroidectomized but uninjected guinea pigs were used as controls. An analysis was also made of the orbital tissues of nine animals in which injection of the hypophyseal extract failed to produce an exophthalmos or in which the proptosis had been transitory and had disappeared at the time of autopsy. In order to provide a control for the extract used, 10 thyroidectomized guinea pigs were injected with extracts of either heart, kidney, or spleen. These extracts were identical in method of preparation and administration with those of the hypophyseal tissue. Six uninjected thyroidectomized guinea pigs were added to the group which were injected with the control extracts.

The extract used to produce an exophthalmos was prepared from the anterior lobe of beef pituitary glands by the method described earlier (10). Fifteen to 20 mgm. of this preparation were injected subcutaneously per day for 60 to 70 days. All of the injected animals were thyroidectomized 10 to 20 days prior to the first injection. The post-thyroidectomy period of the uninjected control animals was the same as that of the guinea pigs injected with pituitary gland extract.

The average and range of body weights of the animals in the experimental and control groups were strictly comparable with the exception of those guinea pigs to which control extracts were given.

METHODS. Since the amount of orbital muscle and fat in the guinea pig

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is very small, the methods used to determine their lipid and water content necessarily differed from standard procedures. The animals were killed with illuminating gas, immediately decapitated, and bled so that extremely little or no blood remained in the orbital vessels. The orbital fat and the extraocular muscles, including the levator, were removed and placed in small glass stoppered weighing bottles. The fat, after any excess fluid had been absorbed with filter paper, was rapidly spread over the bottom of the bottle in a thin layer. The muscles were scattered singly on the walls of the bottle. The bottles and their contents were immediately weighed and then dried *in vacuo* over phosphorus pentoxide at 40°C. until the weights were constant (± 0.1 mgm.). After the drying of the orbital fat the lipid was extracted with six or seven changes of anhydrous ether. The residue was then dried, weighed, and the extractions repeated. Due to the toughness of the fat no fragments were dislodged and lost in the ether extraction. Following the second series of extractions, the residue was dried to a constant weight and the loss in weight caused by the removal of the ether soluble material was taken as the weight of the lipid content of the fat. The largest component of the residuum was connective tissue.

Since the amount of fat and muscle available from a single orbit was very small, duplicate analyses were impossible. However, determinations of the water and lipid content of the tissues from the right and left orbits of the same animals were found to agree closely, so that the methods used are believed to be adequate.

In order to discover whether the changes induced by the injection of anterior pituitary extracts were limited to the orbital tissues, the water content of the peritoneal fat and of skeletal muscle was determined. The fat used in these experiments was taken from the dorsal aspects of the peritoneal cavity, around the adrenals, kidney, ureters and from the uterine mesentery. The mass of peritoneal fat was spread out thinly in a Petri dish, weighed, and then dried in the oven at 100°C. Approximately 20 grams of muscle, including most of the musculature of a fore and hind limb, the abdominal wall, and the psoas and scalene muscles were used. These muscles were rapidly freed of surrounding fat and cut up coarsely with scissors. They were weighed in a Petri dish and dried at 100°C. until the weight was constant (± 25 mgm.).

The degree of exophthalmos was determined, in addition to inspection and palpation of the globe in the living animal, by measuring, at autopsy, the protrusion of the globe from the orbit. After the head had been skinned and severed from the body, the distance from the limbus corneae to the edge of the bony orbit, at the supra-orbital notch, was measured with vernier calipers (the skull-limbus measurement).

RESULTS. Orbital tissues. The injection of extracts of the anterior pituitary glands produced a marked exophthalmos, as determined by the increase in the skull-limbus measurement from 3.8 mm. in the thyroidectomized control animals to 5.6 mm. in the exophthalmic animals (table 1)². The weight of the orbital

² Ablation of the thyroid gland caused a slight increase (0.6 mm.) in the skull-limbus measurement. This effect has been consistent and is more marked in guinea pigs thyroidectomized for very long periods.

fat and muscles of the latter was increased 115 per cent ($P < 0.01$)³ and 33 per cent ($P < 0.01$) respectively. Data from the control series show that thyroidectomy or injection of the control extracts had no effect on the weight of the orbital fat or on that of the extraocular muscles. There was, however, an increase of 39

TABLE 1

Comparison of water and lipid content of the orbital tissues of control and exophthalmic guinea pigs

NUMBER OF CASES	SKULL-LINECS	BODY	ORBITAL FAT				EXTRA-OCULAR MUSCLES			
			Total	Water	Lipid	Residue	Total	Water	Residue	
A. Normal control group										
17	mm.	grams	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	%	mgm.
ϵ_{3f}	0.09	23	2.5	1.6	1.6	0.3	2.2	1.7	0.2	0.6
B. Thyroidectomized, uninjected control group										
26	3.8	529	47.2	29.4	14.1	3.7	81.4	66.1	81.2	15.3
ϵ_{3f}	0.12	19	2.8	1.8	1.5	0.2	2.5	2.1	0.2	0.5
C. Thyroidectomized, injected with anterior pituitary extract, exophthalmic										
19	5.6	545	92.7	70.1	15.4	7.2	104.8	87.2	83.1	17.6
ϵ_{3f}	0.15	18	5.3	5.1	1.0	0.4	2.9	2.6	0.3	0.5
D. Thyroidectomized, injected with anterior pituitary extract, non exophthalmic										
9	3.6	538	60.2	40.0	14.5	5.7	92.5	75.6	81.7	16.9
ϵ_{3f}	0.08	38	3.2	2.2	2.1	0.3	4.6	3.8	0.2	0.9
E. Thyroidectomized, injected with control extracts										
Kidney										
3	3.4	453	39.0	25.9	9.8	3.3	76.3	62.2	81.5	14.1
Heart										
4	3.5	407	37.7	27.9	6.2	3.5	68.5	56.2	82.0	12.4
Spleen										
3	3.5	417	42.4	31.2	7.3	3.9	81.6	66.5	81.4	15.1
Uninjected										
6	3.8	429	36.7	25.6	7.9	3.1	75.1	60.9	81.0	14.1

$$\epsilon_{3f} = \sqrt{\frac{\sum d^2}{n(n-1)}}$$

per cent in the weight of the orbital fat and of 17 per cent in the weight of the extraocular muscles of the animals receiving anterior pituitary extract, but which did not become exophthalmic (table 1).

“P” represents the probability that the difference between the two means is due to random sampling.

The water content of the orbital fat increased from 24.8 mgm. in the normal control animals to 70.0 mgm. in the exophthalmic animals. This tissue normally contains a large amount of water, but in the exophthalmic animals water accounts for 75 per cent of the weight. The water content of the extraocular muscles of these animals was also increased (37 per cent, $P < 0.01$). Ablation of the thyroid gland even when no extracts were injected caused some increase of doubtful significance (4.6 mgm.) in the water content of the orbital fat but none in the muscle tissue. Injection of the control extracts had no effect on the amount of water in either the orbital fat or muscles. However, the water content of the orbital fat of the animals which received pituitary extract, but which failed to become exophthalmic, was slightly increased over that of the thyroidectomized control animals (36 per cent).

The data show clearly that the injection of the anterior pituitary or of the control extracts caused no increase in the lipid constituent of the orbital fatty tissue. In the control extract injected series it will be noted that the amount of lipoidal material is less than that from the animals in the untreated control groups (table 1 A, B and E.). This is presumably due to the use of smaller animals as recipients of the control extracts.

The amount of fat tissue residue after the removal of water and lipids was increased from 3.3 mgm. in the normal controls to 7.2 mgm. in the exophthalmic animals. This increase was consistent and was highly significant statistically. The weight of the dry residue of the ocular muscles was also increased (15 per cent, $P < 0.01$) in the exophthalmic animals, but the extent of the increase was much less than that of the water. There was no increase in the dry tissue residue of either the orbital fat or muscle as a result of thyroidectomy or the injection of control extracts. Since both water and solid components increase in amount in normal muscle hypertrophy, the proportion of water in the extraocular muscles of the exophthalmic and control animals was determined. The data (table 1) show that the eye muscles of exophthalmic guinea pigs contained a higher percentage of water than did those of the various control animals. The difference in water content per unit weight, although small, was very consistent and therefore has a high degree of significance ($P < 0.01$).

Non-orbital tissues. In contrast to the hypertrophy of the orbital fat caused by the injection of the pituitary extract, a decrease in the weight of the peritoneal fat was observed (table 2). Injection of the control extracts or thyroidectomy without the injection of extracts, however, did not change the amount of this fat nor the proportion of water in it relative to that of the normal controls.

The peritoneal fat of the pituitary extract-injected exophthalmic animals contained 55 per cent more water than did similar fat of the thyroidectomized controls (table 2 C). This increase, though definite ($P < 0.01$), was much less than the increase of 138 per cent found in the water content of the orbital fat. There was no change in the water content of typical skeletal muscle (table 2) of these animals. The mean values obtained suggest that thyroidectomy followed by the injection of the hypophyseal extracts tended to increase the

water content. However, statistical analysis reveals that none of the differences is significant. This is in direct contrast to the marked increase in water in the eye muscles. It is noteworthy that the normal eye muscles contained more water than did the skeletal muscles with which they were compared. Injection of the control extracts was without effect on the skeletal muscle water content,

TABLE 2

Comparison of water content of the non-orbital tissues of control and exophthalmic guinea pigs

NUMBER OF CASES	BODY	PERITONEAL DEPOT FAT		BODY MUSCLE WATER
		Total	Water	
A. Normal control group				
11	grams 565	grams 15.2	grams 1.7	per cent 77.9
ϵ_M	34	2.4	0.2	0.4
B. Thyroidectomized, uninjected control group				
18	521	16.2	1.8	78.3
ϵ_M	21	1.8	0.1	0.3
C. Thyroidectomized, injected with anterior pituitary extract, exophthalmic				
16	517	10.9	2.8	79.2
ϵ_M	18	1.1	0.3	0.4
D. Thyroidectomized, injected with anterior pituitary extract, non exophthalmic				
9	538	11.7	2.1	77.8
ϵ_M	38	2.0	0.6	0.6
E. Thyroidectomized, injected with control extracts and uninjected controls				
Kidney				
3	453	7.5	1.9	78.7
Heart				
4	407	7.2	0.9	79.6
Spleen				
3	417	6.4	1.0	80.1
Uninjected				
6	429	6.9	1.3	80.2

as shown by comparison with the appropriate controls. The slightly higher values obtained in this group were due, it is believed, to the use of younger and smaller animals (table 2 E).

DISCUSSION. The data obtained in this study show that the increased weight of the orbital fat of exophthalmic guinea pigs is due almost entirely to an excessive accumulation of water in this tissue. This increased weight of the orbital fat, therefore, is not the result of a true hypertrophy, since the main

cellular constituent, the fat cells, remains unchanged in amount as judged by the weight of the ether soluble lipids. Since there was no increase in lipids, it may be assumed that the framework of the fat cells did not increase in amount. Histological studies have shown an increase in the connective tissue in the orbital fat of exophthalmic animals; therefore it seems probable that the increase in weight of the "residue" fraction of the orbital fat tissue was almost entirely the result of connective tissue proliferation.

Since there was no increase in the total weight of the peritoneal fat of the exophthalmic animals, the high percentage of water in this tissue must have been obtained at the expense of either the lipid or connective tissue framework. These changes were without effect on body weight. The increase in water content of the peritoneal fat suggests that the edema of the orbital fat in experimental exophthalmos is an extreme example of a condition which is, however, not entirely restricted to the orbit.

The water content of the peritoneal fat was more variable than that of the orbital fat. This may have been due in part to the greater range in weight of the peritoneal fatty depots, since the smaller fat deposits contained proportionately more water than did the larger ones. This relationship is illustrated by the data on the water content of the peritoneal fat of the two uninjected thyroidectomized control groups (table 2 B and E). It is also evident that the water content of the orbital and peritoneal fatty tissue of normal guinea pigs differs greatly (peritoneal fat 11 per cent water and orbital fat 57 per cent water). A structural basis for this difference exists, for examination of sections of these tissues has shown that the orbital fat of normal and thyroidectomized guinea pigs possesses a large amount of loose connective tissue which contains an appreciable quantity of interstitial fluid. The fatty tissue which lies about the kidney and in the uterine mesentery, however, has very delicate septa, containing no observable interstitial material.

Hypertrophy of the extraocular muscles has been found to occur in both clinical and experimentally produced exophthalmos. Histological examination has shown that the eye muscles from such cases were extremely edematous (3, 6, 13). The present data demonstrate that nearly all of the increase in weight of the eye muscles of exophthalmic guinea pigs was due to an increase in the water component. However, there was a slight increase in the dry muscle residue, which suggests that some of the water increase was simply the result of muscle growth. This is in agreement with those experiments in which it has been demonstrated that the proptosis itself causes a hypertrophy of the eye muscles, presumably in response to the greater work required of them as retractors of the bulb (11). However, the percentage of water content was higher in the eye muscles of the exophthalmic guinea pigs, indicating that the increase in weight of these muscles was not entirely due to a simple growth hypertrophy.

Since no similar change in the percentage of water in the skeletal muscles was found, the edema of the eye muscles in exophthalmos appears to be a more localized reaction than the similar change in fat tissues. It is noteworthy

that the data on water content of skeletal muscles were based on pooled specimens of many muscles, some of which may have had an increased water content, as did the eye muscles, but which were insufficient in mass to affect the total value. There are at present no histological data available which suggests that the pathological state of the eye muscles in experimental exophthalmos represents a more general condition, excepting a reference by Paulson (8) to changes in the orbicularis oculi of exophthalmic guinea pigs. Examination of sections of diaphragmatic muscles of exophthalmic guinea pigs in our experiments has not revealed any abnormalities.

It is presumed that the cause of the increase in water content is the same in both fat and muscle. An increased vascular permeability may possibly be the factor immediately responsible for it. That contraction of Müller's orbital muscle or growth of the harderian gland does not cause the edema by interference with the vascular drainage of the orbit has been shown in earlier experiments (12). The finding that the water content of non-orbital tissues is also increased may be correlated with clinical observations of localized edema in non-orbital tissues of persons with exophthalmos (2, 4, 5).

CONCLUSIONS

1. Injection of an extract of anterior pituitary glands into thyroidectomized guinea pigs causes an exophthalmos and a hypertrophy of the orbital fat, which is almost entirely due to an increase in the water content of this tissue.

2. No change occurs in the lipid content of the orbital fat of these exophthalmic animals.

3. There is a marked increase of connective tissue in the orbital fat of exophthalmic guinea pigs.

4. The hypertrophy of the extraocular muscles which occurs in experimentally produced exophthalmos is largely caused by an increase in the water component. Some of the increase in water, however, is apparently due to normal growth.

5. The percentage of water found in pooled specimens of skeletal muscle of exophthalmic guinea pigs is not significantly greater than that in muscles from normal and thyroidectomized control guinea pigs.

6. In contrast to the orbital fat, the peritoneal fat depots do not increase in weight in exophthalmic animals, but the injection of anterior pituitary extract into thyroidectomized guinea pigs causes an increase in the water content of the peritoneal fatty tissues.

7. The orbital fat contained much more water than the peritoneal fat in normal and thyroidectomized guinea pigs.

8. Ablation of the thyroid gland had no effect on the weight of the extraocular muscles, orbital and peritoneal fat, or on the water content of the muscles and peritoneal fat, but did cause a slight increase in the water component of the orbital fat tissue.

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THE ABSENCE OF PHOSPHATE TRANSFER IN OXIDATIVE MUSCULAR CONTRACTION¹

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When striated muscle contracts in the presence of an inadequate oxygen supply, the accumulation of lactic acid is accompanied by a loss of phosphocreatine (PC) and an accumulation of inorganic phosphate. At high rates of contraction there is also an accumulation of hexosemonophosphate (HMP). The amount of adenosine triphosphate (ATP) usually does not change. In mammalian muscle contracting with normal blood supply, these phosphate changes apparently stop when the oxygen supply becomes adequate (3, 11).

The Embden-Meyerhof formulation of phosphorylating glycolysis, based on studies of enzyme systems in cell-free extracts, postulates a large number of phosphate interchanges between these compounds and numerous others which have been found only in such extracts, as integral parts of the formation of lactic acid. An alternative formulation has been presented (9) in which only the phosphate changes actually observed are considered to take place. According to this, the hydrolysis of the potassium salts of PC furnishes alkali to buffer the lactic acid, and the formation of HMP is interpreted as a secondary mechanism for supplying energy under anaerobic conditions when the glycolytic mechanism is insufficient.

Still a third formulation has recently been proposed by Needham et al. (6). This is based on the finding by Engelhardt and Ljubimova (2) that myosin acts as an enzyme in hydrolyzing ATP to adenosine diphosphate and inorganic phosphate. Needham and his co-workers have attempted to link the chemical and physical processes in contraction, by postulating the formation of myosin phosphate in this reaction, with the myosin in the extended state. In the contraction processes, the nerve impulse is considered to cause the liberation of inorganic phosphate from the myosin compound, with return of the myosin to the contracted state, and corresponding contraction of the muscle fibril. The ATP is then restored by some phosphate donor. Several possible donors are mentioned, but since PC is the only one present in quantity, this must be the ultimate source of phosphate for this resynthesis. Some other unspecified reaction process must then resynthesize the PC from inorganic phosphate.

Obviously, the over-all changes in the contracting muscle do not give any information on possible intermediate steps. On the other hand, before either the Embden-Meyerhof or Needham formulation can be accepted as valid, it is desirable that direct evidence for such interchanges of phosphate groups be found in the intact muscle. The use of radioactive phosphorus (P^{32}) as a tracer offers

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the means of determining whether such interchanges do take place, if conditions can be found in which the resting metabolism of muscle results in a differential distribution of P^{32} among the various organic compounds. If the Embden-Meyerhof cycle is operating in contraction, then stimulation should result in equalization of the distribution of P^{32} among the three organic compounds; if the mechanism postulated by Needham is active, an equalization should take place between the PC and ATP. On the other hand, if neither set of phosphate interchanges is involved in the contraction process, stimulation should not effect any such equalization in the distribution of the P^{32} .

In previous experiments using the tracer technique (8) it was found that a prolonged tetanic contraction under essentially anaerobic conditions did not affect the distribution of the P^{32} . However, evidence was obtained (10) that the course of resting muscle metabolism does involve interchanges of phosphate groups between PC, the two labile groups of ATP, and inorganic phosphate. It was therefore concluded that the phosphorylating glycolysis should be referred to the resting metabolism of muscle rather than to the metabolism of anaerobic contraction.

These findings do not exclude the possibility of such phosphate interchanges during oxidative contraction, without any over-all changes in phosphate distribution. To test this possibility, the effect of a prolonged series of single twitches on P^{32} distribution was determined. The experiments were performed on cats under pentobarbital anesthesia. The solution containing the P^{32} in the form of Na_2HPO_4 was injected subcutaneously, and the stimulation performed two hours later. Previous work (10) had shown that this time is optimum for obtaining a differential distribution of the P^{32} . One gastrocnemius muscle was subjected to 120 to 180 isometric twitches, by condenser discharges through the nerve, at the rate of 1, 2, or 3 per second for 1 or 2 minutes. This muscle and the companion resting muscle were then frozen, and the phosphate compounds isolated from trichloroacetic acid filtrates by the methods used previously (10). Measurements of relative radioactivity were made by a Geiger-Müller counter. The effect of the stimulation on the contents of inorganic phosphate, PC, ATP and HMP was also determined. In the table below these data are given, with the figures for P content of the stimulated muscles corrected for the uptake of water from the blood stream during the stimulation period. This was done by multiplying the observed values in milligrams per cent, by the ratio of total acid-soluble P of the resting and stimulated muscles.

The results show no evidence for phosphate transfers resulting from the stimulation. In every case in which the resting muscle shows an unequal distribution of P^{32} between any two of the organic compounds, this unequal distribution is retained in the companion stimulated muscle. The only changes in P^{32} distribution are those indicated by the changes in distribution of the total P. Thus neither in anaerobic contraction nor in contraction under oxidative conditions is there any evidence for either the Embden-Meyerhof or the Needham cycle of phosphate interchanges. These data add considerable weight to the conclusion previously indicated (10), that there is a qualitative difference in the

pathways of carbohydrate utilization in resting metabolism and in contraction. Other data in support of this view are the finding (7) that the point at which iodoacetic acid inhibits lactic acid formation in anaerobic contraction is different from that at which the reactions in extracts are inhibited, and the important

TABLE 1

Effect of repeated isometric twitches on distribution of P^{32} and of total P in muscles of cats

In each space the upper figure represents radioactivity, as counts per minute per milligram of P. Lower italicized figure gives P content in milligrams per cent. R = Resting muscle. S = Stimulated muscle.

CAT NO.	INORGANIC P	PHOSPHO- CREATINE	ADENOSINE TRIPHOSPHATE (2 LABILE GROUPS)	HEXOSEMONO- PHOSPHATE	STIMULATION	
					Rate per second	Duration minutes
1 R	565 24	99 70	156 37	260 9		
1 S	352 35	108 37	181 23	243 9	1	2
2 R	530 23	51 49	161 42	83 12		
2 S	385 47	82 26	157 44	72 14	1	2
3 R	392 17	42 61	145 26	89 10		
3 S	262 36	34 50	87 27	47 10	1	2
4 R	258 27	23 61	26 38	147 10		
4 S	130 41	34 39	33 28	95 15	2	1
5 R	505 18	84 58	73 38	107 10		
5 S	321 49	103 18	64 38	100 30	3	1

observation by Stannard (12) that azide inhibits completely the excess oxygen uptake by frog muscle resulting from contraction, in concentrations which do not affect the oxygen uptake of resting muscle.

In the previous experiments (8) no account was taken of the possible difference in P^{32} content of extracellular and intracellular inorganic phosphate. Attention has been called to this by two reviewers (4, 5). However, the essential conclu-

sion indicated in that paper is not invalidated, for there too it was found that contraction did not modify the P^{32} distribution between any two of the organic compounds. The study of resting muscle with this tracer (10) has shown that inorganic phosphate enters into the intracellular inorganic phosphate only by hydrolysis of PC or ATP formed at the membrane. Hence the level of P^{32} in the intracellular inorganic P does not exceed that in PC or ATP. The interpretation there given has been strengthened by the results of Bollman and Flock (1). They found that in rats treated with P^{32} the resynthesis of PC in recovery from contraction did not raise the P^{32} level of the PC beyond that of the resting muscle. The very high P^{32} content of the muscle inorganic phosphate is therefore ascribable to the extracellular phase and the unavoidable inclusion of some blood in the muscle sample.

In agreement with the finding of Bollman and Flock, the present data show no evidence for an increased uptake of P^{32} during the stimulation period. This point again argues against the view that the phosphate interchanges are a fundamental part of the metabolism of contracting muscle.

The point of view here presented does not in any way invalidate the experimental finding of Engelhardt and Ljubimowa that myosin is the enzyme which hydrolyzes ATP. Since there is obviously a breakdown and resynthesis of ATP in resting metabolism, the experimental data show that such an enzyme is operating in resting metabolism. However, this observation, which seemed at first to offer a link connecting the chemical and physical processes in contraction, fails to meet the test of experiment.

The radioactive phosphorus used in these experiments was supplied by the Department of Physics of the University of Michigan.

SUMMARY AND CONCLUSIONS

1. The effect of a prolonged series of twitches on interchanges between the phosphate compounds of muscles has been studied by means of radioactive P.

2. Such contractions, under essentially oxidative conditions, do not bring about interchanges between phosphocreatine, adenosine triphosphate, or hexosemonophosphate.

3. Under these conditions, the contraction process does not result in any increased exchange of intracellular phosphate groups with plasma inorganic phosphate.

4. The evidence obtained indicates that neither the Embden-Meyerhof phosphorylating glycolysis nor the enzyme function of myosin are concerned directly with the chemistry of muscular contraction.

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THE PERSISTENCE OF HEAT ACCLIMATIZATION IN MAN¹

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When man is subjected to high environmental temperature a series of physiological adjustments occurs which reduces the debilitating effects of the heat. The major adjustments take place during the first few days in the heat (1). Heat acclimatization is obviously lost in a cool environment but the rate at which this happens seems to be little known. A survey of the available literature has failed to reveal any reports specifically concerned with this problem. The persistence of heat acclimatization is of importance in many practical situations, notably in military operations.

This paper is a report on 4 series of experiments involving 24 men who were studied at high temperatures on two occasions from 1 to 4 weeks apart. The environment, physical work, and diet were rigidly controlled.

Subjects. The subjects were normal young college students free from physical defects that might influence cardiovascular adjustments. Ages ranged from 19 to 28 years.

The subjects remained continuously in the air-conditioned suite at the Laboratory from the afternoon preceding each exposure to high temperatures to the end of each experiment. During the 1 to 4 week intervals between exposures to the heat the subjects were not at the Laboratory. The experiments were performed during the winter months so that the subjects encountered high temperatures only during the experiments.

PROCEDURE. A standard regimen of observations, physical work, rest and diet was maintained in the 4 series. The length of the intervals between exposures to the heat was 1 week in series I, 2 weeks in series II, three weeks in series III and 4 weeks in series IV.

For each experiment the routine consisted of work during two successive afternoons at 78°F. followed by two full days of work at temperatures of 110°F. in the mornings and 120°F. in the afternoons. Night temperature was 85° to 90°F. Relative humidity was held constant at 20 to 25 per cent saturation.

The physical work consisted of walking on a motor-driven treadmill at a constant rate of 3.25 miles per hour and a 7.5 per cent angle of climb. Work of this intensity demands an oxygen consumption at about 7 times the basal rate. Each half day's work consisted of 6 ten-minute work periods alternating with

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ten-minute rest periods. Standard clothing of cotton shorts, shoes and socks was used for work and one-piece U. S. Army Armored Force battle dress (cotton drill) was used during the evenings.

Pulse rates were counted before and during the first 15 seconds immediately after each work period. Rectal temperatures were taken with a clinical thermometer for the first 90 seconds after the third and sixth work period each morning and afternoon. Cramptom (2) "blood ptosis" tests were made before and after each days' work. Body weights were recorded each morning before breakfast and with an empty bladder. The subjects were weighed to $\pm \frac{1}{4}$ ounce nude and dried before and after the third work period each morning and the sixth

TABLE 1

The grand averages of the physiological variables measured in the four series of experiments for the 1st and 2nd exposures to the heat

ROOM TEMP.	WORK PULSE RATE-BEATS/ MIN.					RECTAL TEMPERATURE, °F.					CRAMPTON SCORE*				
	78° F.	110° F.	120° F.	110° F.	120° F.	78° F.	110° F.	120° F.	110° F.	120° F.	78° F.	90° F.	120° F.	90° F.	120° F.
	p.m.	a.m.	p.m.	a.m.	p.m.	p.m.	a.m.	p.m.	a.m.	p.m.	p.m.	a.m.	p.m.	a.m.	p.m.
<i>Series I</i>															
1st exposure..	112	134	145	126	136	100.3	99.9	100.5	99.6	100.4	71	71	56	64	59
2nd exposure..	109	127	132	127	126	100.2	99.7	100.1	99.5	99.9	67	58	65	70	63
<i>Series II</i>															
1st exposure..	109	132	139	122	140	100.1	99.7	100.4	99.8	100.4	68	66	44	54	62
2nd exposure..	116	123	123	117	125	99.9	99.6	99.9	99.6	100.1	66	77	52	69	66
<i>Series III</i>															
1st exposure..	117	141	151	135	155	100.1	100.6	101.1	100.5	101.5	75	76	46	64	50
2nd exposure..	112	140	149	134	142	100.2	100.3	101.0	100.3	100.8	69	71	49	64	64
<i>Series IV</i>															
1st exposure..	116	138	140	129	137	99.9	100.3	100.5	100.2	100.4	79	64	44	60	64
2nd exposure..	113	136	139	128	142	100.1	99.8	100.3	99.6	100.4	63	65	46	54	61

* A higher score means a better adjustment.

work period each afternoon for rates of sweating. Twenty-four hour urine output and fluid intake volumes were recorded for the two days in the heat.

A constant diet was provided by a trained dietitian for three days preceding and during each experiment. The diet contained about 3,100 calories and was normally balanced in carbohydrate, protein and fat. It was adequate according to current standards in all the vitamins and provided 15 ± 2 grams of sodium chloride daily. Water was allowed *ad lib*.

RESULTS. The grand averages for the physiological variables measured and the statistical evaluation of the mean (t-values, Goulden, 3) are given in tables 1 and 2. Each half day's pulse rate represents the average of from 24 to 48 individual work pulse rate counts. Similarly the rectal temperature values for each half day represent the average of 12 to 24 determinations.

The work pulse rates were significantly lower on the second exposure for both the first and second afternoons in the heat in series I and II. In series III the

advantage of having been partially heat acclimatized three weeks previously showed up only on the second afternoon in the heat. For the four week interval (series IV) there was no hold-over effect. The morning work pulse rates in the heat and the afternoon values at normal temperatures (78°F.) were not significantly different between the first and second exposures in any of the series.

The rectal temperatures exhibit the same progressive decline in hold-over effect as do the work pulse rates. The rectal temperature differences are significant for both the first and second afternoons in the heat when the interval between exposures is only one week (series I). The two and three week interval groups (series II and III) are improved on either the first or second afternoon in the heat but the four week group (series IV) was not improved on the second exposure.

In general the Crampton test demonstrated no benefit derived from a previous exposure to heat except on the first afternoon in series I. Even though the *t*-

TABLE 2

t-values for the differences between the means of the physiological variables for the two exposures to the heat on the 1st and 2nd afternoons

A negative *t*-value indicates a higher mean obtained on the second exposure to the heat

	WORK PULSE BEATS/MIN.		RECTAL TEMP., °F.		CRAMPTON SCORE		t-VALUES FOR	
	1st p.m.	2nd p.m.	1st p.m.	2nd p.m.	1st p.m.	2nd p.m.	1% level	5% level
Series I.....	5.613	7.752	3.279	3.333	-2.388	-1.01	3.50	2.36
Series II.....	3.543	6.122	4.10	1.957	-0.959	-0.364	4.60	2.78
Series III.....	2.05	5.312	0.653	5.035	-0.421	-1.879	3.71	2.45
Series IV.....	0	1.935	0.649		-0.452	0.322	5.84	3.18

values are not significant there is, however, a progressive trend toward a lower *t*-value as the interval between the two exposures to heat is greater. The Crampton test has previously been found to be a less sensitive index of the state of acclimatization than are the work pulse rates and rectal temperatures (1, 4).

The grand averages for the observations on water balance during work in the heat are given in table 3. There was no significant difference or trend in the rate of sweating during work, fluid intake, urine output or weight loss between the first and second exposure to the heat in any of the 4 series. The absolute values are about the same as those observed by the authors in other studies when the work output and temperatures were comparable. It is, however, interesting that in series I and II the calculated total sweat volume was nearly 1½ liters less during the second exposure to the heat. In series III it was only slightly decreased while in series IV it was ½ liter greater.

DISCUSSION. We have noted the absence of previous reports on the persistence of heat acclimatization. In the popular press there have been statements that German troops destined for hot weather service in the African deserts were "conditioned" in heated buildings in Germany. From the present results it is

clear that such preparation might be of real value even though as long as several weeks elapsed between acclimatization in Germany and the operation in Africa.

TABLE 3
Average values for water balance during the two days in the heat

	RATE OF SWEATING, GRAMS/MIN.				WATER INTAKE	URINE OUTPUT	BODY WEIGHT CHANGE	CALCULATED TOTAL SWEAT*
	110°F., a.m.	120°F., p.m.	110°F., a.m.	120°F., p.m.				
					liters	liters	ounces	liters
<i>Series I</i>								
1st exposure.....	9.3	12.6	8.1	12.6	9.713	1.669	+6	8.700
2nd exposure.....	9.5	11.9	10.8	13.6	9.841	2.300	+32½	7.390
<i>Series II</i>								
1st exposure.....	9.6	11.6	10.2	12.2	10.110	2.477	+22	7.722
2nd exposure.....	8.2	13.1	9.1	12.3	6.845	1.630	-4½	6.343
<i>Series III</i>								
1st exposure.....	11.7	13.2	13.2	14.6	9.937	1.707	-12½	9.382
2nd exposure.....	12.1	13.3	13.3	13.7	11.009	2.829	+7½	8.839
<i>Series IV</i>								
1st exposure.....	9.8	12.5	11.2	13.3	9.539	2.350	+10½	7.640
2nd exposure.....	9.7	11.2	10.1	12.2	9.842	2.156	+17	7.952

* Total sweat = (water drunk + water in food + water of metabolism) - (urine output + water in feces + water in expired air) ± (body weight change during the two days in the heat).

TABLE 4

The number of individuals showing a change in physiological variables between the two exposures to the heat on the 1st and 2nd afternoons of work

	WORK PULSE RATE CHANGE GREATER THAN 5 BEATS/MINUTE			RECTAL TEMPERATURE CHANGE GREATER THAN 0.2°F.			GRAMPTON SCORE CHANGE GREATER THAN 5		
	Increase	No change	Decrease	Increase	No change	Decrease	Increase	No change	Decrease
<i>Series I</i>									
1st p.m. heat.....	0	1	7	0	2	6	4	3	1
2nd p.m. heat.....	0	1	7	0	1	7	5	3	0
<i>Series II</i>									
1st p.m. heat.....	0	0	5	0	0	5	2	2	1
2nd p.m. heat.....	0	0	5	0	2	3	2	2	1
<i>Series III</i>									
1st p.m. heat.....	0	7	0	2	1	4	4	2	1
2nd p.m. heat.....	0	0	7	0	0	7	6	1	0
<i>Series IV</i>									
1st p.m. heat.....	1	2	1	1	0	3	2	1	1
2nd p.m. heat.....	3	1	0	2	0	2	1	1	2

We have no information as to the persistence of acclimatization after a first exposure of less than or more than the 2-day period used here but it may be presumed that a longer exposure might result in somewhat more persistent ac-

climatization. Another question of interest would be the possible accumulation of ability to acclimatize to the heat through repeated exposures.

Individuals differ markedly in their response to work in heat but individual variations were not prominent in these studies on persistence of heat acclimatization. Table 4 gives the number of individuals in each series who showed a change in the physiological variables between the first and second exposures to heat. "Real" differences were arbitrarily considered to be changes of 5 beats per minute for work pulse rate, 0.2°F. for rectal temperature and 5 points for the Crampton score. Work pulse rate is the most sensitive index of the condition of the subject in the heat. The uniformity of behavior of this variable in the several subjects is notable in the first 3 series. The rectal temperature is less reliable and the Crampton test is the least consistent variable studied.

The detailed nature of the changes in acclimatization to heat is unknown but they are exhibited largely in cardiovascular effects and the principal components are the heart, the blood volume, and the constriction and dilatation of the appropriate blood vessels. Presumably these are of increasing importance in the order named. Accordingly it would seem that the autonomic nervous system dominates the picture.

SUMMARY

1. The performance of muscular work in dry heat was studied in 24 normal young men. The diet, environment and work routine were carefully controlled during the stay in the heat.

2. Comparisons were made between the performances in the heat on two occasions of 2 days each separated by from 1 to 4 weeks of cold weather.

3. Work pulse rates, rectal temperatures and, to a lesser degree, vasomotor stability tests showed that heat acclimatization persists during at least three weeks of cold weather. The benefit conferred by this acclimatization is important for this time but the advantage decreases from week to week.

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SOME EFFECTS OF PECTIN SOLUTIONS DURING POSTHEMORRHAGIC HYPOTENSION¹

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The use of an autoclaved pectin solution as a blood substitute has been advocated by Hartman and associates (1) on the basis of its colloidal osmotic pressure and its capacity to restore effective circulating volume and arterial pressure after hemorrhage and experimental procedures causing shock.

This report is concerned (a) with the question whether such solutions administered after standard periods and degrees of posthemorrhagic hypotension prevent the development of an irreversible state, and (b) with an analysis of hemic and hemodynamic actions following their use under such conditions.

Preparation. A 1.5 per cent solution of autoclaved pectin kindly supplied by Frederick Stearns & Co., Detroit, was used in these experiments. Various shipments were stated to have a viscosity of 3.58 to 4.86, and osmotic pressures between 67 and 68.7 cm. H₂O. The micellar aggregate size was about 60,000. In some experiments solutions with a pH of 3.68 to 3.9 were brought to 6.9 to 7.2 by phosphate buffers recommended by Hartman et al.; in other experiments a buffer supplied with the pectin and containing glycine, lactate and sodium hydroxide was added in recommended amounts. These brought solutions to a pH of 6.6.

PART I. *The substitute value of pectin solutions.* Since conclusions as to the substitute value of a solution depend not only on its properties but also on the conditions under which it is tested, the procedure used and the reasons for its adoption must be briefly stated.

Dogs anesthetized with morphine and biologically standardized doses of sodium barbital (2) were bled at a rate of 50 ml./min. until mean arterial pressure was reduced to 50 mm. Hg. Any tendency to increase was neutralized by bleeding a little more from time to time. In this way, a 50 mm. hypotension period was maintained in different animals from 30 to 120 minutes. If no infusion was given after 30 minutes, arterial pressures failed to recover. If, however, the animal's own blood (heparinized) was infused after 90 minutes of such hypotension, 90 per cent of animals recovered. Indeed, the small percentage of deaths can probably be accounted for by failure fully to prevent certain hazards in the drawing and keeping of such blood (inadequate heparinization, faulty filtration, etc.). Not only was aortic pressure restored but the pressure pulses maintained a normal form for 6 to 8 hours after such infusions. With such

¹ This investigation was supported by a grant from the Commonwealth Fund.

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actions of a presumably ideal transfusate after large hemorrhages, the effects of autoclaved pectin solutions were compared.

The procedure was chosen 1, because previous experiments (3) indicated that any damaging influence of severe hemorrhage is correlated better with the degree and duration of the hypotension induced than with the quantity of blood withdrawn, even when bleeding occurs at constant rates, and 2, because it afforded variable periods for development of the damaging effects of a standard hypotension. Furthermore, since a good blood substitute should not merely restore and maintain the effective circulating volume and arterial pressure, but should also prevent the development of an irreversible state under conditions in which the animal's own blood does so, this method seems to offer a test which is more nearly comparable with situations arising in practice than procedures in which blood is immediately replaced after a hemorrhage. It seems highly improbable that, in practice, a large hemorrhage can be checked and a transfusion given in less than the minimal interval of 30 minutes used in these experiments.

Results. In various experiments pectin infusions were given at a rate of 3-5 ml./min. after existence for 30, 50, 90 or 120 minutes of a 50 mm. post-hemorrhagic hypotension. Even faster rates of infusion were tolerated by normal animals without adverse effects, as revealed by optical pressure curves and electrocardiograms. Nor were gross pathologic disturbances seen in any organ at autopsy.

Thirty-two dogs, exclusive of controls, were used. In 19 of these, the volume of pectin solution practically equaled the volume of blood withdrawn; in 7 it was about 65 per cent of the blood lost. In 6 dogs, 80 per cent of the blood was replaced by pectin and this was *immediately followed* by an infusion of 20 per cent of the animal's own heparinized³ blood.

Of the 32 dogs, 10 recovered from pectin infusions, 17 died from a precipitate type of circulatory failure and 5 after a delayed failure. A "breakdown" of these results is of interest. In 1 experiment the infusion was started after two hours of 50 mm. hypotension; in 3, after 90 minutes and in 7, after 40 to 49 minutes. All of these animals died quickly. In 3 animals which developed a precipitate shock following pectin infusions, the animal's blood was reinfused. This only revived the animals temporarily. This is additional evidence that an irreversible state had developed despite infusion of pectin solutions. On the other hand, of the 19 animals in which infusion began after 30 minutes of hypotension, a satisfactory dynamic recovery of the circulation occurred in 11 while a precipitate or delayed circulatory failure occurred in 8 dogs. It seems apparent that the usefulness of autoclaved pectin solutions after severe hemorrhage is restricted to the early periods of hypotension which follow.

For this reason, only the latter experiments merit further analysis. Details of these are given in table 1 and plots of representative experiments illustrating circulatory recovery, delayed and precipitate circulatory failure are shown in figures 1, 2 and 3. In 6 experiments in which smaller blood transfusions followed

³ We are indebted to Roche-Organon, Inc., Rahway, N. J. for the Liquaemin used in these experiments.

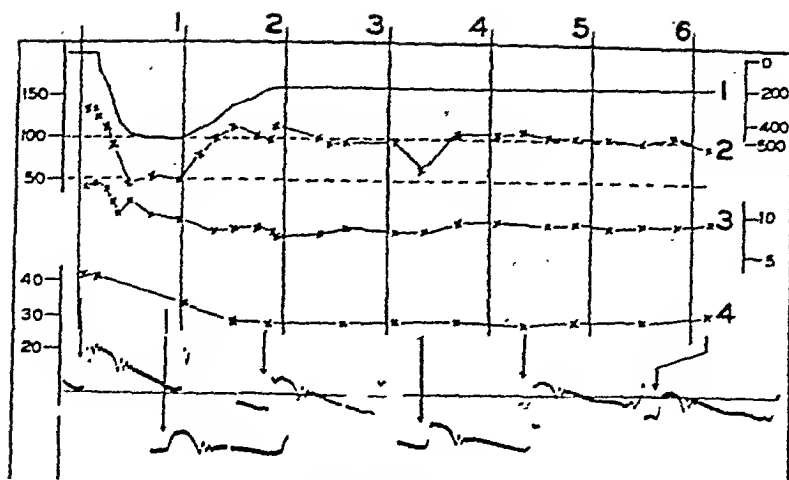


FIG. 1

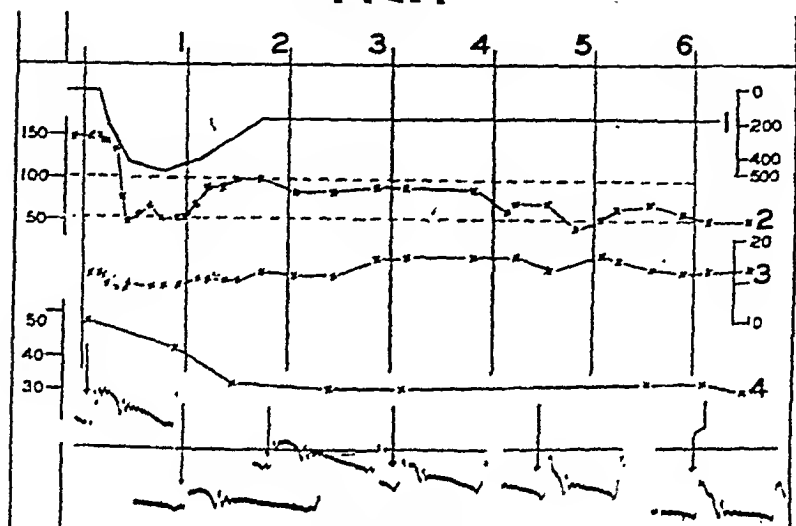


FIG. 2

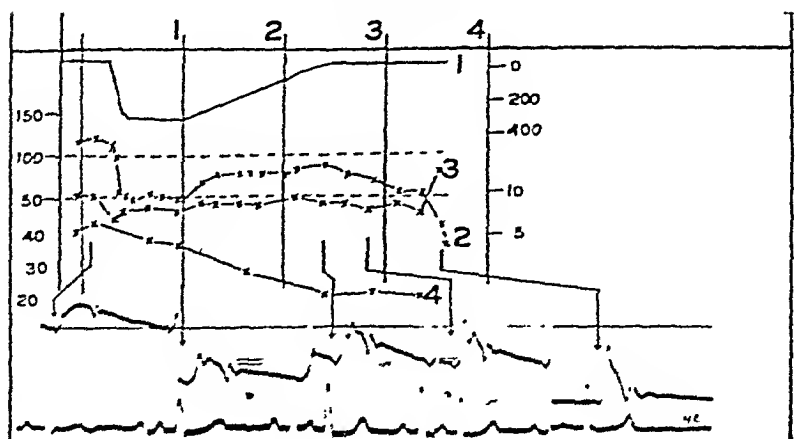


FIG. 3

Fig. 1. Circulatory recovery.

Fig. 2. Delayed circulatory failure.

Fig. 3. Precipitate circulatory failure. Plots in all indicate 1, amount and rate of blood withdrawn (downstroke) and volume and rate of pectin infused in milliliters; 2, mean arterial pressure in millimeters of mercury; 3, effective venous pressure in centimeters saline; 4, hematocrit readings as percentage of red cells. Below segments of central pressure pulses taken at times indicated by arrows and in figure 3 an e.c.g.

use of pectin certainly no incompatibilities developed; on the contrary, these infusions appeared to be beneficial.

PART II. *Hemic effects of pectin solutions.* Small samples of arterial blood were drawn periodically to study blood changes. Rapid sedimentation rates and pseudoagglutination (microscopic and macroscopic evidence of clumping) were found in blood mixed with pectin in the body as well as in vitro. However, no postmortem evidence of stasis, congestion, infarction, etc., was found in survival animals. Animals that went into shock usually revealed duodenal mucosal changes which were similar to but paler than those of animals dying without such infusions (3).

As shown in the plots of figures 1, 2 and 3, the arterial hematocrit readings were reduced after a 30 minute period of hypotension, as anticipated. In a few cases (expts. 2, 13, 14, 15, fig. 1, columns 1, 2) the decrease may not have been significant and in two instances (expts. 1, 18) an apparent concentration occurred. These few exceptions bring up the question whether stock animals used for such experiments are always in an adequately hydrated state. In three animals (expts. 6, 7, 8), the spleen was exteriorized and while emptied of its blood by a small dose of epinephrine, its pedicle was clamped. In all of these animals, hemodilution occurred after hemorrhage, indicating that this organ is not responsible for changes observed after hemorrhage. These observations are sufficient to indicate that at the time pectin solutions were injected, a state had not been reached when fluid was leaving the circulating system; on the contrary, the flow of water seemed to be toward the blood stream.

The effects on hematocrit readings 3 to 5 minutes after recirculation of the infused pectin and at the end of 4 to 6 hours are shown in figures 1, 2 and 3 and in columns 3, 4 of table 1. The first serves as a rough index of the effective dilution due to the injected solution itself; the latter gives some idea as to osmotic capacity for attracting water exerted by colloidal pectin a few hours after infusion. In each of the experiments in which pectin only was injected (expts. 1-13) a tremendous additional dilution occurred at once. Red cell volumes decreased 13.9 to 19.3 per cent in all except the three splenectomized animals in which they decreased only 6, 8.6 and 8.4 per cent respectively. Curiously, an equal or even greater reduction occurred in the five experiments in which an infusion of some blood followed use of pectin solutions (expts. 15-19).

Many plots similar to those of figure 1, 2 and 3 showed, with few exceptions, that no further reduction in hematocrit values occurred, and at the end of 4 to 6 hours they increased gradually. Of course, the readings were much less than at the beginning (cf. columns 1 and 4, fig. 1). The significance of such observations must be tempered by the knowledge that animals under morphine and barbital ordinarily show a progressive hemoconcentration of even greater magnitude (4). However, if injected pectin solutions exert any osmotic attraction for tissue fluids after hemorrhage this must be small. These reactions following severe hemorrhage are apparently different from those observed on normal animals and man (1, 5). On the other hand, the degree of dilution achieved by its infusion seems to be maintained for 4 to 6 hours.

A review of our results shows, however, that the persistence of low hematocrit readings does not correlate with recovery of blood pressure in our experiments; on the contrary, animals which showed the greater tendency to return to normal concentrations were the ones that recovered (cf. expts. 1, 2, 4, 5, 13, 18, with expts. 7, 8, 9, 10, fig. 1). While no relation of increasing hemoconcentration to recovery can be claimed, the results strongly suggest that the ability of a solution to maintain hemodilution cannot be used as a sole criterion of its dynamic usefulness.

TABLE 1

EXP.	HEMOR- RHAGE	DURA- TION 50 MM. HYPO- TENSION	PECTIN INFUSION	BUFFER	HEMATOCRIT				OUTCOME
					1	2	3	4	
	<i>ml./kilo</i>		<i>ml./kilo</i>						
1	30	35	30	Phosphate	39.0?	45.7	26.4	33.4	Recovery
2	38	31	38	Phosphate	46.7	42.3	28.4	33.7	Recovery
3	47	35	47	Phosphate	54	51.7	51.7	31.3	Recovery
4	46	29	46	Phosphate	49.0	43.0	27	37	Recovery
5	42	31	40	Phosphate	51	48	22	35	Recovery
6*	38	30	23	Phosphate	42.7	33.5	27.5	30.0	Recovery
7*	28	32	28	Phosphate	45.2	34.1	25.5	21.2	Precip. shock
8*	5.5	30	11	Phosphate	40.5	38.3	29.9	29.7	Precip. shock
9	32	34	33	Phosphate	42.4	36.8	23.1	20.9	Precip. shock
10	40	32	25	Phosphate	49.3	42.3	29.5	28.2	Precip. shock
11	36	31	32	Phosphate	58.5	52	34.5	38.5	Precip. shock
12	33	29	29	Phosphate	53	39	25	36	Precip. shock
13	33	31	33	Lactate	41.5	40	27	42	Recovery
14	40	30	20 + 20B	Lactate	47	40	31	36	Recovery
15	52	37	33 + 4B	Lactate	47.5	46.5	19	34	Recovery
16	33.5	33	40 + 10B	Lactate	50	46	22	34	Recovery
17	53	31	26 + 6B	Lactate	46.5	35	25	26.5	Delayed shock
18	39	25	32 + 6.4B	Phosphate	37?	41	26	33	Recovery
19	41	30	31 + 8B	Phosphate	41	35	24	34.5	Delayed shock

* Splenectomized dogs. Hematocrit readings—(1) control, (2) at end of 50 mm. period hypotension, (3) after infusion and recirculation of pectin, (4) 4-6 hours later.

PART III. *Electrocardiograms and hemodynamic reactions.* Electrocardiograms (standard leads) were recorded during the course of most experiments. Segments of one of these are incorporated in figure 3. No changes of significance occurred either in animals that recovered or those that failed. During the period of hypotension all deflections decreased in amplitude and during terminal slowing of the heart the P-R interval sometimes increased (fig. 3). In particular, no electrocardiographic evidence was obtained which suggested thrombosis or impairment of the coronary or pulmonary circulations. Optical records of central arterial pressure pulses were recorded in 8 animals.

Segments of such pressure pulses obtained in animals responding differently to pectin infusions are shown in figures 1, 2 and 3. In figure 1, from a dog which made a satisfactory hemodynamic recovery, an infusion of pectin solution

equal to 65 per cent of the withdrawn blood was given. Mean arterial pressure was not fully but satisfactorily restored and it was maintained during the period of observation. The pulse pressure following infusion was somewhat less than during the control period, but the form of the pressure curve was normal and remained so for 4 hours. Incidentally, this experiment illustrates during the third and fourth hour a frequent occurrence in such revived animals, viz., a temporary decline of arterial pressure, 60 to 90 minutes after completion of infusion. This was always followed by a secondary rise which was maintained. During such temporary drops the form of the pressure pulses did not deteriorate, and electrocardiograms showed no significant changes.

Figure 2 illustrates a type of experiment in which infusion of pectin solution was followed by a delayed circulatory failure. Immediately following injection the form of the pressure pulses was restored to normal. Gradually, this deteriorated, the curves being characterized by a large preliminary fling, a peaked systolic summit, deep incisura and progressively flattening diastolic part of the curve. These changes resemble those previously reported as characteristic of circulatory failure (3). It may be noted that in this experiment, as in others previously reported, circulatory failure developed despite gradually increasing effective venous pressure.

Figure 3 illustrates one of the experiments in which reinfusion of full amounts of pectin solution failed to restore the form of pressure pulses even temporarily and a subsequent rapid deterioration. It will be noted that in this experiment the rate of infusion was slower, but similar precipitate shock also occurred in experiments in which a more rapid rate was used.

DISCUSSION. The data presented strongly suggest that infusions of pectin solutions are ineffective after a 30 minute period of a 50 mm. Hg post-hemorrhagic hypotension. Given at the end of 30 minutes, 11 out of 19 dogs showed satisfactory hemodynamic reactions. This series is too small to venture opinions on a statistical basis, but certain reactions, particularly in animals that did not respond favorably, suggest caution in the use of such solutions after severe hemorrhage. It should be emphasized that our deductions do not necessarily apply to states of hypotension produced otherwise than by loss of blood and particularly when these are accompanied by hemoconcentration.

We have confirmed many observations of previous workers, considered adequate for their clinical trial. Thus, after a post-hemorrhagic hypotension of not too long duration, their infusion causes an immediate increase in pulse pressure, restoration of arterial pressure and generally normal forms of pressure pulses. Hematocrit readings suggest that hemodilution occurs by virtue of the fluid injected and that a good degree of retention occurs for at least 4 to 6 hours. Evidence that blood volume is further increased by virtue of an osmotic attraction of water from tissues is lacking. However, this may be a virtue rather than a fault, for an excessive depletion of interstitial and cellular water may be harmful rather than beneficial after hemorrhage. Recent evidence (6) indeed suggests that cellular water is already utilized in the hemodilution which follows hemorrhage. However, no correlation existed between maintenance of an initial

dilution of blood by pectin and hemodynamic recovery which suggests that it is hazardous to rely on the demonstrated ability of pectin solutions—or for that matter any colloid—to remain within the blood stream as a satisfactory criterion of its efficacy. Hemodynamic recovery depends on more than the capacity of an infusate to exert an adequate oncotic pressure.

While no direct evidence was obtained either experimentally or at the necropsies that the agglutination and rapid sedimentation is harmful, the occurrence of a rapid decline in arterial pressure and deterioration of pressure pulses in some of the experiments and the failure to overcome this by subsequent large infusions of the animal's own blood certainly does not suggest that infusion of pectin solution delays the development of an irreversible circulatory state. It is conceivable, as Ivy et al. (7) suggest, that a tendency to intravascular clumping may reduce oxygen supply more seriously after severe hemorrhage than in animals having their normal quota of red corpuscles. Further experimentation, however, is required to establish this or other reasons for the too common unfavorable responses.

SUMMARY

1. A 1.5 per cent buffered pectin solution exerting an oncotic pressure of 67 to 68.7 cm. of water was injected at a rate of 3–5 ml. per minute after varying periods of post-hemorrhagic hypotension (50 mm. Hg).

2. The usefulness of such solutions is apparently limited to the early periods of such hypotension. Given after 30 minutes, 11 out of 19 dogs showed a satisfactory hemodynamic response for 4 to 6 hours after administration.

3. Pectin infusions, given after a post-hemorrhagic dilution of blood has already occurred, cause a further dilution by virtue of the fluid injected and this is rather well maintained for 4 to 6 hours. Evidence that additional dilution results from osmotic attraction of water from tissues was not found.

4. No correlation existed between maintenance of hemodilution and favorable hemodynamic reactions; on the contrary, the animals that recovered usually showed some tendency to reconcentration. This suggests that the demonstrated ability of a colloid *a*, to maintain an effective oncotic pressure over a considerable time interval, and *b*, to increase blood volume in normal animals is not a satisfactory criterion of its physiological usefulness.

5. While no evidence was obtained experimentally or at necropsy that the rapid sedimentation and agglutination produced by such solutions is harmful, the occurrence of a precipitate failure of the circulation in too many experiments and the inability to overcome this by subsequent large infusions of blood suggest that pectin infusions may exert some deleterious influence when used after severe hemorrhage. Consequently, caution should be exercised in the employment of pectin solutions in such conditions.

6. These conclusions do not necessarily apply to states of hypotension produced otherwise than by loss of blood and particularly when hemoconcentration exists.

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THE RENAL EXCRETION OF CHLORIDE BY THE NORMAL AND BY THE DIABETES INSIPIDUS DOG¹

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The only obvious abnormality of the diabetes insipidus animal is its excessive excretion of water; otherwise the renal function of such an animal, insofar as it has been studied (19, 25), is apparently normal. The question, however, remains, whether this inability to reabsorb water is the fundamental deficiency of the diabetes insipidus renal tubule, or whether it is merely secondary to some alteration in the tubular reabsorption of solutes. The possibility of a disturbance in chloride excretion has been investigated many times, but the continuation of these experiments indicates that no decisive answer has been obtained. This lack of success was probably due to the concentration of most investigators on excretion alone, and it is extremely difficult to evaluate their data, since excretion is the net result of at least two variables, glomerular filtration and tubular reabsorption.

Some electrolytes are reabsorbed like glucose from the glomerular filtrate in definitely limited quantities. If the glomeruli, because of increased volume of filtrate or of an elevated plasma concentration, deliver such a substance to the tubules in an amount greater than the tubular capacity for reabsorption, the excess is excreted in the urine; phosphates and sulphates belong to this group. The maximal rate of tubular reabsorption of phosphate in the dog is 0.8 to 4.2 mgm./min., but is dependent upon the acid-base balance of the animal (9), and is subject to vitamin and hormonal control (10). Goudsmit, Power and Bollman (7) showed that sulphate clearances asymptotically approach the simultaneous creatinine clearances in dogs as the plasma sulphate is progressively increased. This indicates a low tubular maximum of sulphate reabsorption, and a recalculation of their data reveals the maximum to be less than 10 mgm./min.

A second type of renal reabsorption is comparable to the intestinal reabsorption of salt solution, in which the volume of fluid and the quantity of electrolyte transferred are closely related. When a solution of sodium chloride is placed in a loop of gut, the salt is absorbed along with most of the water, until a chloride-free fluid is left in the intestinal lumen. Visscher (22) has pointed out the similarity of this process to the renal tubular reabsorption of electrolyte, where the parallel is especially close during water diuresis and in diabetes insipidus. If the disturbance in water reabsorption in diabetes insipidus is secondary to an abnormal electrolyte reabsorption, or more specifically chloride reabsorption, it is probable that a mechanism of this kind is involved.

Our primary problem was to establish the behavior of chloride in the normal kidney, since this is extremely ill-defined and is essential to any hypothesis of an

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abnormal process in diabetes insipidus. We soon found that there was no apparent limit either to the amount of chloride excreted per minute or to its concentration in the excreted urine. We were also unable to establish any general relationship between chloride excretion and plasma chloride values. When we turned our attention to reabsorption, we found that there was no apparent tubular maximum for chloride, or, if there was such a limit, it was not attained when the quantity of chloride filtered was increased as much as fourfold. If, however, the quantity of chloride reabsorbed was compared with the quantity of water reabsorbed in the same time, a very definite relationship was apparent, and one which was consistent from experiment to experiment and from one dog to another.

This relationship, the ratio of reabsorbed chloride to reabsorbed water, is not intended to express anything as simple as the concentration of the reabsorbed solution. Walker et al. (23), in studies on single mammalian nephrons, have shown that the glomerular fluid soon attains, as it progresses down the tubule, a chloride concentration about 1.4 times that of plasma. Therefore, chloride and water seem to be reabsorbed in different parts of the tubule, and the ratio of reabsorbed chloride to water is merely the resultant of these reabsorptive processes. This ratio is not a constant, but is related to changes in plasma chloride, and it was soon found that a comparison of the chloride:water ratio in the reabsorbate with the chloride concentration of the plasma was a useful expression of the reabsorptive process; it was designated the chloride R/P.

When saline solutions of different concentrations were infused at the same rate into a normal dog in successive experiments, it was found that a family of curves resulted from relating the chloride R/P to time. The configuration of each curve was characteristic of the concentration of the saline infused. When the experiments were repeated on a dog with diabetes insipidus, the responses were unpredictable and not related to the concentration of the injected fluid. The chloride R/P of the diabetes insipidus dog is initially greater than one and may rise considerably further during saline infusion, reaching a maximum value in our experiments of 1.2. Since the addition of Pitressin to the infusion fluid altered these responses until they approximated those of the normal animal, it was concluded that the neurohypophysis regulates the partition of water between tubular reabsorbate and urine. This is true, of course, only of that fraction of the water, about 20 per cent, which is not reabsorbed by the diabetes insipidus tubule; the remaining 80 per cent of water filtered is reabsorbed by the tubule even in the complete absence of the posterior lobe. Our conclusion is based on data obtained from more than one hundred experiments on three normal dogs and four dogs with experimental diabetes insipidus.

PROCEDURES. Experimental diabetes insipidus was produced by transection of the pituitary stalk. Our usual procedure (8) for creatinine clearances was followed, with these modifications: venous blood was drawn under oil into oxalated tubes for centrifugation; and fluids, other than water by stomach tube, were administered intravenously with a constant infusion pump. In the earlier experiments, plasma and urine chlorides were determined by the method of Van Slyke and McLean (16), but later the simpler mercurimetric titration (18) was

adopted, after it had been established that analyses by the two methods were almost identical. Glucose, in tungstic acid filtrates of plasma and in diluted aliquots of urine, was determined by measuring with a photoelectric colorimeter the reduction of an alkaline ferricyanide solution.

Calculations. The volume of fluid reabsorbed per minute by the tubules was calculated by subtracting the urine flow in cubic centimeter per minute from the creatinine clearance; reabsorbed chloride by subtracting the quantity excreted per minute from the quantity filtered. This last value was obtained by multiplying the volume of glomerular filtrate by the milligrams of chloride per cubic centimeter of plasma. The ratio of chloride to water in the reabsorbate, $\frac{\text{mgm. Cl}}{\text{cc. H}_2\text{O}} \times 100 =$ mgm. per cent reabsorbed, was then compared to the plasma chloride according to the equation: Chloride R/P = $\frac{\text{mgm. per cent reabsorbed Cl}}{\text{mgm. per cent plasma Cl}}$.

The correction factor for the solid content of plasma has not been applied in these calculations, since it did not significantly affect the chloride R/P value.

RESULTS. The earlier experiments were performed to obtain data for analysis in the hope of revealing factors which limited chloride excretion or reabsorption. They were done, therefore, on normal dogs and included water diuresis, intravenous infusion of glucose and hypo-, iso- and hypertonic saline solutions. Three of these experiments, in which different concentrations of saline were infused, are presented in table 1. These data have been analysed in several ways. The lack of correlation between urine flow and chloride excretion is obvious on inspection of table 1. However, a nearly linear relationship exists between the quantity of reabsorbed water and of reabsorbed chloride (fig. 1). When 10 per cent glucose or 0.2 to 0.3 per cent NaCl solutions were infused, and the plasma chloride concentration lowered, the curve was altered as less chloride was reabsorbed per unit volume of water (fig. 1). If the plasma chloride concentration is greatly elevated by the infusion of 2.0 to 5.0 per cent NaCl, the chloride:water ratio of the tubular reabsorbate is definitely increased (fig. 1). This deviation of the chloride:water ratio at low and high plasma concentrations indicates its dependence upon changes in the salt content of the plasma. The chloride R/P was found to change during the course of each experiment in which hypo- or strongly hypertonic saline solutions were given. When the data in table 1 are analysed to show the temporal course of the chloride R/P, three distinct curves are obtained (fig. 2). While it appears from this figure that the contour of each curve is related to the concentration of the salt solution injected, other variables, such as the rate and duration of the infusion, are not excluded as influencing factors. Six infusions into the same normal dog, L, were constant except for the concentration of the solution, which was 0.55, 0.67, 1.06, 1.5, 2.0 and 2.5 per cent NaCl. When the chloride R/P is related to time (fig. 3), a progressive modification of the response with increasing concentrations of NaCl is apparent.

The next part of this investigation is concerned with the regulating mechanism which so exactly controlled the partition of water between urine and reabsorbate that when hypotonic solutions were given, little salt and large volumes of water

were excreted, and when strong saline was infused, large quantities of salt were excreted in highly concentrated urine. The neurohypophysis was naturally given first consideration.

When the subject was a dog with diabetes insipidus, the results were in sharp contrast to the highly integrated responses of the normal dog. Infusions of 2.0 or 2.5 per cent NaCl instead of producing a depression of the chloride R/P, often caused a further rise of the already elevated ratio (fig. 4). The posterior lobe of the pituitary, therefore, seems to be involved in the depression of the chloride

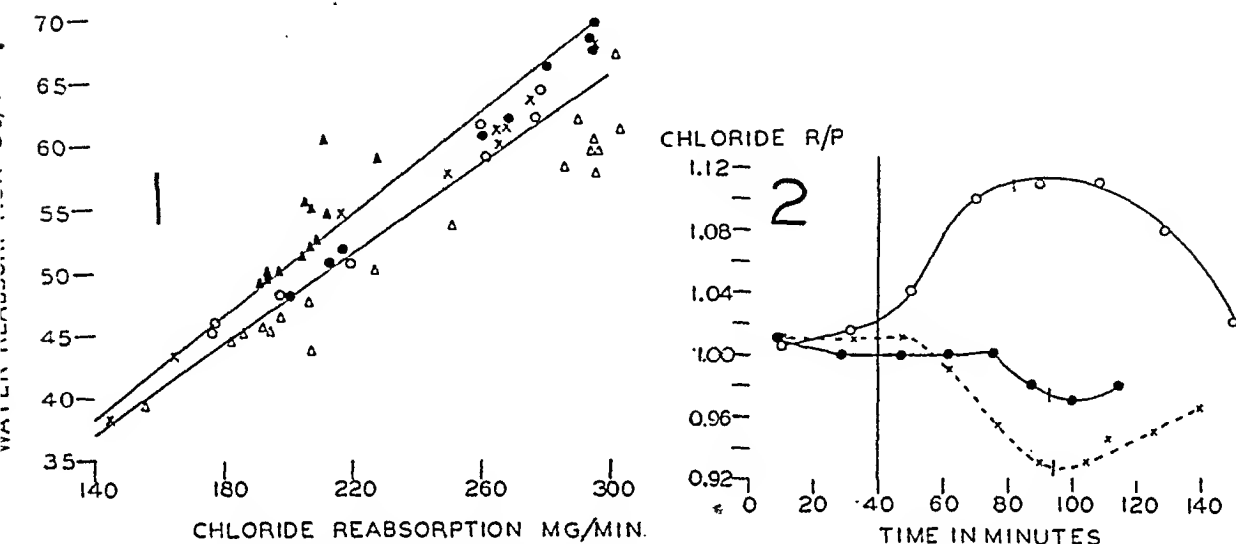


Fig. 1. Chloride reabsorption plotted against water reabsorption. Those data which are taken from table 1 are represented by the following symbols: open circles, 0.5 per cent NaCl; solid circles, 0.685 per cent NaCl; crosses, 2.0 per cent NaCl. Solid triangles represent the data from experiments in which the plasma chloride was reduced by the infusion of 0.2 to 0.3 per cent NaCl or of glucose solutions. Open triangles are data obtained during or after the infusion of 2.5 to 5.0 per cent NaCl solutions when the plasma chloride concentration was greatly elevated.

Fig. 2. Temporal course of the chloride R/P on infusion of 0.5 per cent (open circles), 0.685 per cent (solid circles), and 2.0 per cent NaCl (crosses). Beginning of infusions at 40 minutes is indicated by long vertical line; end of infusions by short vertical lines. Data from table 1.

R/P below one. Attempts to convert the response of the diabetes insipidus dog to normal by substitution therapy with Pitressin (Parke, Davis & Co.) were successful. In a series of infusion experiments, repeated at weekly intervals, 2.5 per cent NaCl was injected at 10 cc./min. for 45 minutes. In the first no Pitressin was given; in the others Pitressin was added to the saline so that it was administered at rates of 60, 180 and 720 milliunits per hour. The results are given in table 2, and the chloride R/P plotted against time in figure 4. While the depression of the chloride R/P with 720 mu./hr. is about as great as that seen in experiments on normal dogs (see fig. 3, lowest curve) receiving the same infusion, there are minor differences in the responses. The response of the normal dog has a latent period of 15 to 30 minutes, measured from the beginning of the infusion, and continues for at least 30 minutes afterward. In the dia-

betes insipidus dog receiving Pitressin, the response begins with the infusion and, at the end of the infusion, falls off in every case more rapidly than the normal, but at a rate which is decreased as the Pitressin dose is increased. The

TABLE 1

Renal response of the normal dog to the infusion of hypo-, iso- and hypertonic sodium chloride solutions

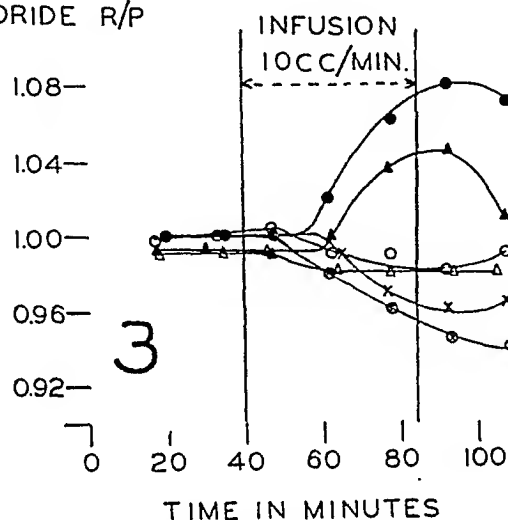
DOG AND INFUSION	PERIOD	DURATION	URINE FLOW	URINE CHLORIDE	PLASMA CHLORIDE	CREATININE CLEARANCE	CHLORIDE (MG./MIN.)			WATER REABSORBED	MG. CHLORIDE REABSORBED 100 CC. WATER REABSORBED	CHLORIDE R/P
							Filtered	Excreted	Reabsorbed			
P 0.5% NaCl 29 cc./min.	1	20	0.56	31.9	385	46.5	178	0.18	178	45.9	388	1.006
	2	20.2	0.73	24.3	385	45.9	177	0.18	177	45.2	391	1.015
	3	21.0	2.91	92.5	395	51.0	201	2.70	198	48	413	1.04
	4	20.2	9.12	155.0	393	59.7	235	15.0	220	50.6	434	1.10
	5	20.4	10.6	134	390	75.0	292	14.2	278	64.4	432	1.11
	6	17.8	8.4	79.5	400	67.3	269	6.7	262	58.9	445	1.11
	7	20.0	7.2	97.0	411	69.0	284	7.0	277	62	447	1.08
	8	21.1	3.5	279	415	65.0	270	9.8	260	61.5	422	1.02
P 2% NaCl, 8.7 cc./min.	1	28.6	0.3	43.3	379	43.5	165	0.13	165	43.2	383	1.01
	2	16.2	0.35	22.4	379	38.3	145	0.08	145	38.0	382	1.01
	3	14.0	0.99	100	395	55.4	218	0.99	217	54.4	399	1.01
	4	15.5	2.67	501	427	54.6	233	13.4	220	51.9	424	0.99
	5	14.6	5.73	672	454	63.5	288	38.5	250	57.8	433	0.955
	6	9.9	7.95	736	478	68.0	325	58.6	266	60.0	443	0.93
	7	19.9	5.28	874	470	66.7	314	46.1	268	61.4	438	0.93
	8	14.1	3.12	982	461	70.8	326	30.6	295	67.7	436	0.945
	9	14.4	2.47	1050	456	66.0	301	26.0	275	63.5	433	0.95
	10	14.3	2.13	930	450	63.5	285	19.8	265	61.3	433	0.965
E 0.685% NaCl, 21.5 cc./min.	1	15.3	0.67	179	415	51.5	214	1.2	213	50.8	419	1.01
	2	14.9	0.63	199	415	52.5	218	1.2	217	51.9	418	1.01
	3	24.4	0.51	200	415	48.5	201	1.0	200	48.0	417	1.00
	4	13.8	0.79	350	429	61.7	264	2.8	261	60.9	429	1.00
	5	14.1	1.30	522	431	63.6	275	6.8	268	62.3	430	1.00
	6	13.0	3.00	626	437	70.6	309	14.4	295	67.6	436	1.00
	7	11.7	3.68	605	436	72.3	315	22.2	293	68.6	428	0.98
	8	14.0	3.43	668	433	70.0	303	22.9	280	66.6	421	0.97
	9	14.0	2.78	688	433	72.5	314	19.1	295	69.7	424	0.98

continued fall of the chloride R/P in the normal dog suggests a continued liberation of the antidiuretic hormone.

During the infusion of 2.5 per cent NaCl and Pitressin, the creatinine U/P falls, even when as much as 720 mu./hr. is injected. This behavior of the cre-

atinine U/P is the opposite of that seen when the antidiuretic hormone is given during a water diuresis or to an animal with diabetes insipidus. Under these conditions an elevation of the creatinine U/P, signifying a greater tubular reabsorption of water, is invariably obtained, and Hickey, Hare and Hare (11) have demonstrated a quantitative relationship between the dose of pituitrin and the response. From the present experiments it is clear that changes in the creatinine U/P during a saline diuresis, whether in a normal dog (fig. 6) or in a diabetes insipidus dog receiving Pitressin (table 2), have no significance and are valueless as indicators of the action of the antidiuretic hormone. In contrast to

CHLORIDE R/P



CHLORIDE R/P

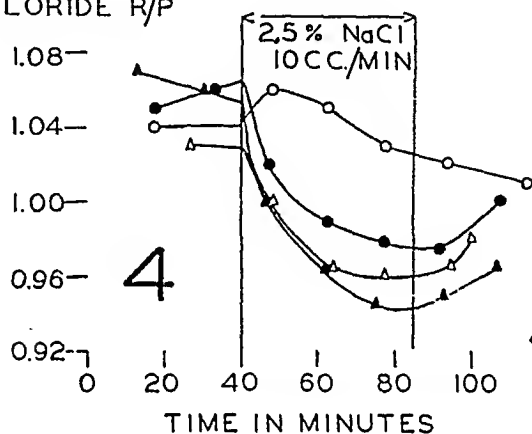


Fig. 3. Temporal course of the chloride R/P during infusions of graded saline solutions into the same normal dog, at 10 cc./min. for 45 minutes. Solid circles, 0.55 per cent; solid triangles, 0.67 per cent; open triangles, 1.06 per cent; open circles, 1.5 per cent; crosses, 2.0 per cent; and enclosed crosses, 2.5 per cent NaCl.

Fig. 4. Relationship of the chloride R/P of a dog (S) with diabetes insipidus to the amount of Pitressin added to the infusion fluid (2.5 per cent NaCl). The vertical lines enclose the infusion period. Open circles, no Pitressin; solid circles, 60 mu/hr.; open triangles, 180 mu/hr.; solid triangles, 720 mu/hr. Data from table 2.

this, the chloride R/P (fig. 4) is related to the amount of antidiuretic hormone introduced into the dog's circulation during a saline diuresis.

An elevation of the chloride R/P is the permanent effect of the destruction of the pars nervosa of the pituitary and the temporary consequence of the ingestion of water or the injection of glucose or hypotonic saline. It seemed probable that these procedures elevated the chloride R/P by inhibiting the liberation of the posterior lobe hormone. If this were true, the administration of small amounts of the hormone should decrease or prevent the elevation of the chloride R/P during the course of such a diuresis. This has been tested repeatedly, and the experiment selected for presentation illustrates the effect of 1.7 and 6.8 mu. of Pitressin per hour on the diuresis resulting from the injection of 0.55 per cent NaCl at 10 cc./min. (fig. 5). The same normal dog, L, was the subject for all three experiments which were as nearly alike as possible. The response of the

animal to hypotonic saline, as indicated by the chloride R/P, is measurably depressed by 1.7 mu. of Pitressin per hour, and completely suppressed by 6.8 mu./hr. The minute quantity of the antidiuretic hormone needed to restore

TABLE 2

The effect of graded doses of Pitressin on the renal excretion of sodium chloride and water by a dog with diabetes insipidus

DOG AND INFUSION	PERIOD	DURATION	URINE FLOW	URINE CHLORIDE	PLASMA CHLORIDE	CREATININE CLEARANCE	CHLORIDE (MG./MIN.)			WATER REABSORBED	MG. CHLORIDE REABSORBED 100 CC. WATER REABSORBED	CHLORIDE R/P
							Filtered	Excreted	Reabsorbed			
S 2.5% NaCl	1	47.1	2.03	59	384	39.9	153	1.2	152	37.9	402	1.04
	2	15.2	3.96	205	420	43.2	181.5	8.1	173.4	39.2	445	1.06
	3	15.4	8.4	345	448	47.6	213	29	184	39.2	469	1.05
	4	14.4	13.5	423	475	59.5	282	57	225	46.0	489	1.03
	5	18.0	10.6	435	478	52.6	251	46	205	42.0	488	1.02
	6	18.7	7.24	441	473	52.2	247	32	215	45.0	478	1.01
S 2.5% NaCl + Pitressin, 60 mu/hr.	1	14.7	2.58	23.7	382	43.8	167	0.6	166.4	41.2	403	1.05
	2	15.5	2.28	21.4	379	39.3	149	0.5	148.5	37.0	402	1.06
	3	15.2	1.96	265	396	43.6	173	5.2	168	41.6	404	1.02
	4	14.3	6.05	467	431	51.5	222	28.3	194	45.5	427	0.99
	5	15.1	12.4	491	458	60.4	277	60.6	216	48.0	450	0.98
	6	14.0	11.4	514	459	60.6	278	58.5	220	49.2	445	0.975
	7	15.1	7.7	460	448	58.1	261	35.2	226	50.4	448	1.00
S 2.5% NaCl + Pitressin, 180 mu/hr.	1	27.4	1.54	66.5	388	34.4	133	1.0	132	32.8	403	1.04
	2	14.0	1.21	400	424	39.6	168	4.85	163	38.4	424	1.00
	3	15.6	4.18	673	460	47.2	217	26.0	191	43.0	444	0.965
	4	14.7	10.85	564	484	56.1	272	61.3	211	45.2	466	0.965
	5	19.7	11.40	566	481	60.6	292	65.0	227	49.2	462	0.96
	6	10.9	8.05	538	471	60.5	285	43.2	242	52.5	461	0.98
S 2.5% NaCl + Pitressin, 720 mu/hr.	1	14.8	3.5	68.5	371	41.6	154	1.95	152	38.1	399	1.07
	2	20.7	2.8	63.5	372	42.2	157	1.8	155	39.4	394	1.06
	3	13.7	0.73	425	396	40.1	159	3.1	156	39.4	396	1.00
	4	14.7	2.0	770	445	45.6	203	15.5	187.5	43.6	430	0.965
	5	13.3	7.6	625	467	53.4	249	47.4	201.6	45.8	442	0.945
	6	15.0	10.9	567	466	58.4	272	61.5	210.5	47.5	444	0.95
	7	15.5	9.1	541	458	58.8	269	49.4	219.6	49.7	442	0.965

and maintain a chloride R/P of unity is again demonstrated in an experiment on a dog with diabetes insipidus. A 1.5 per cent solution of NaCl was infused for 80 minutes at 5 cc./min.; later the experiment was repeated with the addition of Pitressin to the saline so that its infusion rate was 4.25 mu./hr. The results

summarized in table 3 further justify the hypothesis that an elevated chloride R/P is evidence of inhibition of the neurohypophysis.

An application of this thesis, that changes in the chloride R/P reflect changes in the rate of release of the antidiuretic hormone, to a further analysis of the data from the normal dog receiving different concentrations of saline (fig. 3), permits an interpretation of the diuretic action of salt solutions. When the concentration of each saline solution is plotted against its diuretic effectiveness expressed by the minimal creatinine U/P obtained during or after the infusion of the solution, it is clear that very dilute or very concentrated saline are equally effective diuretics (fig. 6). When the maximal change of the chloride R/P is similarly plotted against the salt content of the infusion fluid, different explanations for the diuresis with dilute and with concentrated saline are suggested.

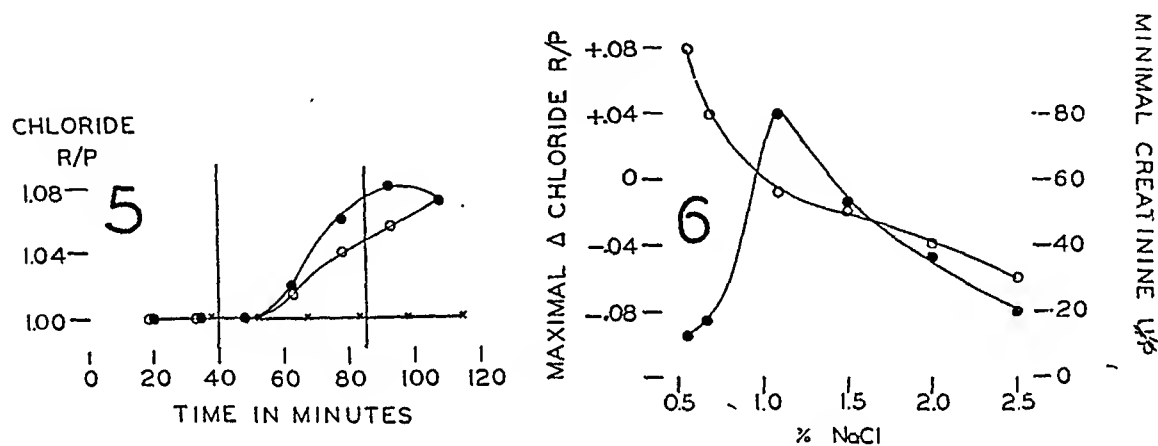


Fig. 5. Inhibition by Pitressin of the elevation of the chloride R/P of a normal dog during the infusion of 0.55 per cent NaCl at 10 cc./min. Vertical lines at 40 and 85 minutes indicate the beginning and end of the infusion period. Solid circles, no Pitressin; open circles, 1.7 mu/hr.; crosses, 6.8 mu/hr.

Fig. 6. The curve drawn through the open circles relates the maximal change in chloride R/P to the concentration of NaCl in the infused solution; that through the solid circles, the minimal creatinine U/P observed in the same experiments.

The copious urine flow resulting from the infusion of 0.55 per cent NaCl is effected through inhibition of the posterior lobe of the pituitary, as indicated by the marked elevation of the chloride R/P. But during the comparable diuresis obtained with 2.5 per cent NaCl, where the depression in the chloride R/P suggests the release of several hundred milliunits of antidiuretic hormone, the volume of urine excreted can be attributed to the large amount of salt excreted. The infusion of 0.9 to 1.5 per cent NaCl neither inhibits the pituitary nor leads to the excretion of sufficient salt to produce an osmotic diuresis; solutions within this range of concentrations have, therefore, the least diuretic effect.

So far, we have centered attention on the changes in the partition of water between urine and tubular reabsorbate and the hypophyseal regulation of this distribution. Since the nature of the calculation for the derivation of the chloride R/P is such that a change in the ratio would be produced by a change in the tubular reabsorption of chloride, we shall now consider this process and the

evidence for its control by the pituitary. When we injected 0.1 to 2.0 mu. of Pitressin into dogs with diabetes insipidus, we regularly obtained a marked decrease in urine flow, but no increase of chloride excretion was observed in any of the ten experiments. Furthermore, we did find the chloride excretion increased three- to fivefold in a normal dog during water diuresis, when the anti-diuretic content of the blood is assumed to be subnormal.

Our experiments on dogs with diabetes insipidus given a constant infusion of 2.5 per cent NaCl solution with graded doses of Pitressin dissociate antidiuretic activity from any demonstrable change in chloride excretion. When the rate of chloride excretion during each experiment is related to time, no significant or consistent variation appears which can be related to the amount of Pitressin

TABLE 3

The control of a saline diuresis in a dog with diabetes insipidus with a small dose of Pitressin

DOG AND INFUSION	PERIOD	DURATION	URINE FLOW	URINE CHLORIDE	PLASMA CHLORIDE	CREATININE CLEARANCE	CHLORIDE (MG./MIN.)			WATER REABSORBED	MG. CHLORIDE REABSORBED 100 CC. WATER REABSORBED	CHLORIDE R/P
							Filtered	Excreted	Reabsorbed			
D 1.5% NaCl, 5 cc./min.		minutes	cc./min.	mgm.%	mgm.%					cc./min.		
	1	20.0	5.01	39.4	400	79.5	318	2	316	74.5	424	1.06
	2	19.6	6.21	128	406	84.0	341	8	333	77.8	428	1.05
	3	20.9	8.22	187	410	86.0	353	15.3	338	77.8	434	1.06
	4	19.7	11.0	181	427	79.8	342	20	322	68.8	468	1.09
D 1.5% NaCl, 5 cc./min., Pi- tressin, 4.25 mu/hr.	1	19.5	1.85	50	397	60.4	239	0.9	238	58.5	407	1.02
	2	19.0	2.06	295	420	69.8	293	6.0	287	67.7	424	1.01
	3	21.2	3.74	442	430	75.8	326	16.5	310	72.1	430	1.00
	4	21.1	5.45	462	444	75.4	334	25	309	70.0	442	0.995

injected (fig. 7); the amount of water excreted is, however, clearly dependent (fig. 8). Additional evidence is included in table 3, where marked antidiuresis was obtained without any such change in chloride excretion. For two reasons these observations are of only limited value as support for the thesis that physiological quantities of the antidiuretic hormone do not act by inhibiting tubular reabsorption of chloride. In the first place, Pitressin, a fraction of pituitary extract, was used, and Kuschinsky and Bundschuh (22) find the principle of pituitrin affecting chloride excretion associated with the oxytocic rather than with the antidiuretic fraction. A second objection is that the dogs were heavily loaded with salt. However, the observations do justify the contention that an antidiuresis can be produced by increasing only the reabsorption of water. Further support for this idea is found in experiments on the diabetes insipidus dog

with a normal salt intake, where Pitressin greatly facilitated the tubular reabsorption of water without affecting chloride excretion.

White and Findley (24) found that patients with diabetes insipidus failed to excrete an oral dose of salt as rapidly as normal persons, and that pituitrin therapy was not effective in correcting this derangement. When we followed the chloride excretion before, during and after similar injections of salt into normal and diabetes insipidus dogs, no consistent difference between the two groups was revealed. On several occasions the polyuric dog excreted more salt than the

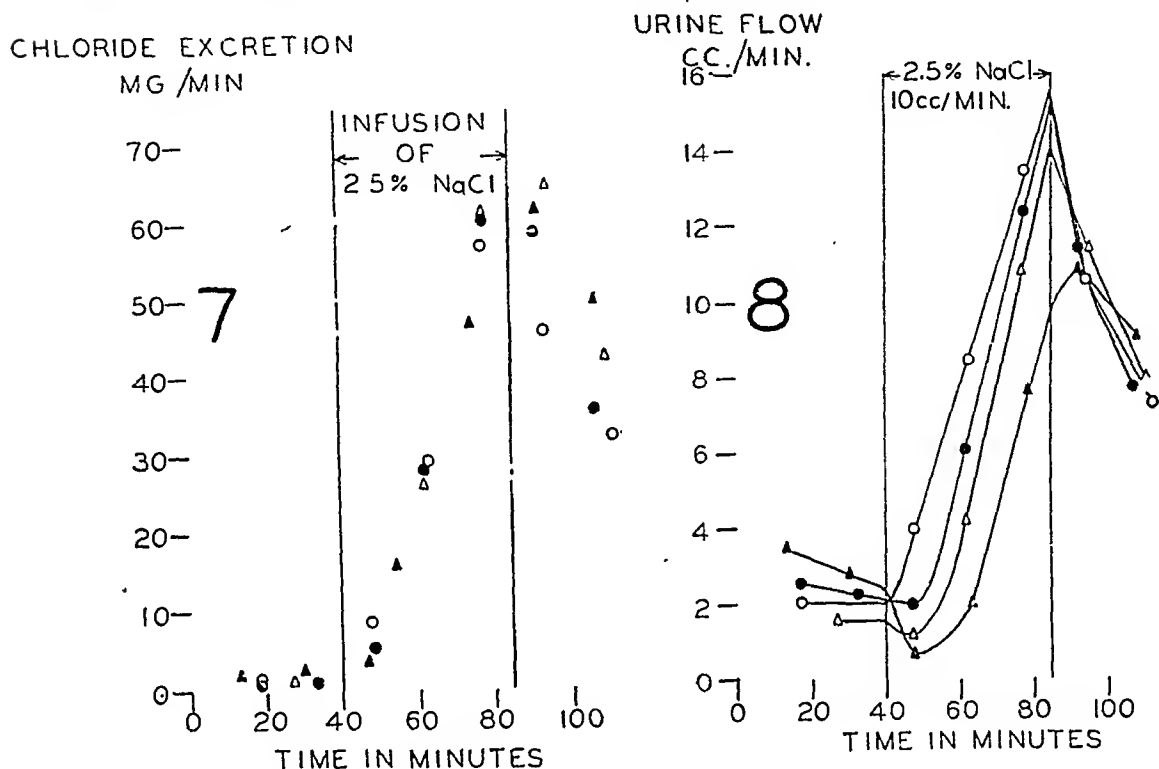


Fig. 7. Independence of chloride excretion of Pitressin administration in a dog (S) with diabetes insipidus. Vertical lines indicate period of infusion of 2.5 per cent NaCl at 10 cc./min. Open circles, no Pitressin; solid circles, 60 mu/hr.; open triangles, 180 mu/hr.; solid triangles, 720 mu/hr. Data from table 2.

Fig. 8. Antidiuretic effect of Pitressin during saline diuresis in a dog (S) with diabetes insipidus. Symbols the same as in figure 7.

normal control. One of these comparisons is presented in detail in table 4 and the first experiment of table 2. The excretion of chloride, plotted against time in figure 9, is greater in the polyuric dog in spite of the fact that glomerular filtration is less. The more rapid rise of the plasma concentration in the diabetes insipidus dogs has been seen repeatedly, and probably has its origin in the excessive water loss of these animals. In this instance the polyuric dog excreted 744 cc. of urine during the experiment which lasted an hour and a half, while the normal dog excreted only 185 cc. during the same time. Probably for the same reason, the polyuric dog developed a severe salt intoxication (26) with tremors, weakness and vomiting. However, the higher rate of salt excretion by the poly-

uric dog has been observed in five experiments of this type when no symptoms of salt intoxication were apparent.

Water diuresis frequently (17, 21), and glucose (2) or sucrose (12) regularly increase chloride excretion. An infusion of 10 per cent glucose at 10 cc./min. may cause an initial tenfold increase in the renal loss of salt (table 5 and fig. 10), but this effect falls off, even during the first few hours, so that the chloride excretion rate becomes only two or three times normal. This elevated excretion occurs during a progressive fall of plasma chloride, and therefore does not have its origin in the animal's chloride load, which Wolf (26) has shown to be a related factor in chloride excretion during chloride infusion. If salt is added to the glucose solution, the renal excretion of chloride proceeds at least as rapidly, or

TABLE 4

Renal response of a normal dog to the infusion of 2.5 per cent NaCl at 10 cc. per minute
Compare with similar experiment of dog with diabetes insipidus, experiment 1, table 2

DOG AND INFUSION	PERIOD	DURATION	URINE FLOW	URINE CHLORIDE	PLASMA CHLORIDE	CREATININE CLEARANCE	CHLORIDE (MG./MIN.)			WATER REABSORBED	MG. CHLORIDE REABSORBED 100 CC. WATER REABSORBED	CHLORIDE R/P
							Filtered	Excreted	Reabsorbed			
		minutes	cc./min.	mgm. %	mgm. %					cc./min.		
L Normal, 2.5% NaCl, 10 cc./min.	1	16.4	0.47	245	389	45.8	178	1.1	177	45.3	391	1.00
	2	11.5	0.82	341	399	47.0	187	2.8	184	46.2	398	1.00
	3	16.8	1.79	742	432	52.5	227	13.3	214	50.7	422	0.98
	4	15.2	2.96	860	458	57.6	264	25.4	239	54.6	438	0.96
	5	16.1	3.12	934	459	61.5	282	29.2	253	58.4	434	0.945
	6	15.1	2.78	1070	454	64.1	291	29.8	261	61.3	426	0.94

even more rapidly than when the same amount of salt is given alone (table 5 and fig. 10). This occurs even when the plasma chloride is still below normal.

Perhaps the most consistent effect of the administration of salt to a dog, whether by mouth or by intravenous injection, is an increase in the rate of glomerular filtration (19). While it is true that the infusion of a large volume of water as a vehicle for glucose (20) may cause an increase in glomerular filtration, the change in our experiments has never been as great as that caused by the infusion of sodium chloride. Furthermore, when 10 per cent glucose has been infused at a constant rate for an hour or more, and the elevated rate of filtration has become stabilized, the addition of salt to the infusion fluid caused a further and sustained rise. This is illustrated in the first experiment of table 5.

DISCUSSION. Walker's (23) studies on the tubular reabsorption of chloride are, unfortunately, incomplete and limited to the proximal convoluted tubule. Until these direct studies have been extended to include the rest of the nephron

and the hormonal factors which regulate reabsorption, only the final result of the various tubular processes can be determined from experiments on the intact animal. The proper choice of an electrolyte or other substance to be associated with the tubular reabsorption of water in the calculation of R/P ratios can be made, of course, only after the tubular transfer of that substance is clearly shown to be an essential process in water reabsorption. The choice of chloride was not made on that basis, and may, therefore, be premature. But the use of the chloride R/P does permit some insight into the response of the normal dog kidney to endogenous antidiuretic hormone, for the temporal course of the chloride R/P

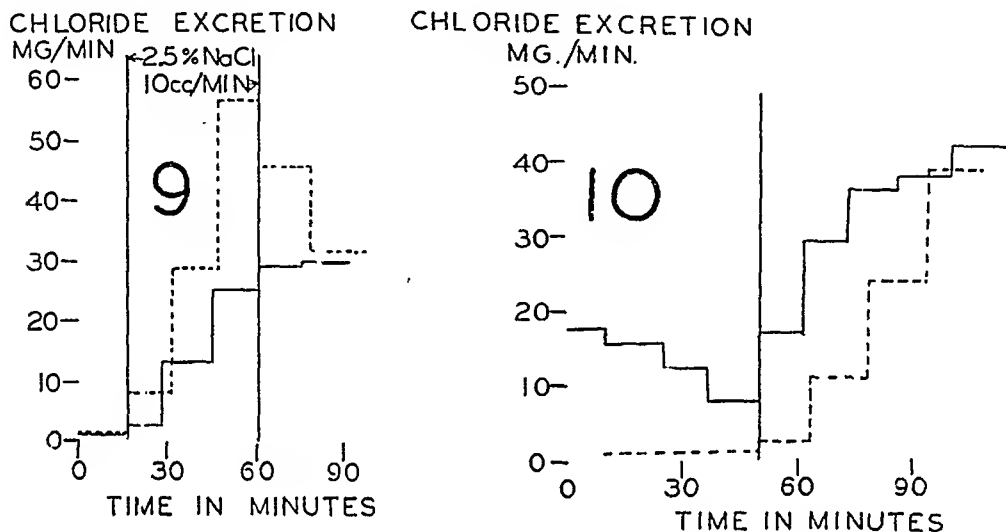


Fig. 9. Comparison of the renal excretion of chloride by a normal (solid line) and a diabetes insipidus (broken line) dog. The vertical lines indicate the infusion of 2.5 per cent NaCl at 10 cc./min. Data for normal dog from table 4; for diabetes insipidus dog from expt. 1, table 2.

Fig. 10. Facilitation of chloride excretion by glucose diuresis. To the left of the vertical line, the excretion of chloride by a normal dog during glucose infusion (solid line) begun two hours before zero time is compared with the basal normal chloride excretion (broken line). At the vertical line, the infusion of 2 per cent saline was begun in both cases; the NaCl was added to the glucose solution so that in both cases the infusion rate was 10 cc./min.

during a saline diuresis has been a reliable indicator of the amount of the hormone discharged by the pituitary or introduced into the circulation by infusion.

Gilman and Goodman (5) discovered that the high rate of urine flow of a normal animal after the administration of 5 per cent NaCl is attended by a greatly augmented liberation and renal excretion of the antidiuretic hormone. They estimated that 8 to 12 rats excreted 100 to 200 milliunits within six hours after being given 5 per cent of their body weight of salt solution by stomach tube. Ingram, Ladd and Benbow (13), using the same methods, found that the antidiuretic material excreted by cats during saline diuresis originated in the pars nervosa of the pituitary. According to their assays, 20 to 420 milliunits per day were excreted by a cat, dehydrated by intravenous injection of 10 per cent NaCl after purging with $MgSO_4$ by stomach tube. We have estimated that a

normal dog of 15 kgm. liberates into its blood stream about one unit of pituitrin when given 450 cc. of 2.5 per cent NaCl by intravenous infusion (figs. 3 and 4). Our data are not comparable in a quantitative manner with those mentioned above, since the latter measured the hormone excreted by a normal animal, while we have measured replacements necessary to reproduce a response in a dog deprived of its source of the hormone. All the data are in agreement, however, that saline, in hypertonic solutions, is extremely effective in causing a release of pituitrin; whether this is a specific effect of NaCl, or simply dehydration, is not clear (11). This excessive liberation of the hormone may well account for the failure of huge doses to inhibit a saline diuresis. Goodman and

TABLE 5

Augmentation of chloride excretion of a normal dog by the infusion of glucose

DOG AND INFUSION	PERIOD	DURATION	URINE FLOW	URINE		PLASMA		CREATININE CLEARANCE
				Chloride	Glucose	Chloride	Glucose	
		<i>minutes</i>	<i>cc./min.</i>	<i>mgm.%</i>	<i>mgm.%</i>	<i>mgm.%</i>	<i>mgm.%</i>	
E *10% glucose at 10 cc./min.	1	14.9	12.2	127	3000	355	910	57.1
	2	11.6	7.6	158	4160	355	850	57.6
	3	13.3	5.5	136	5000	350	710	59.6
	4	11.7	7.7	216	4450	355	800	69.0
10% glucose + 2% NaCl at 10 cc./min.	5	11.8	11.1	262	3270	365	840	66.6
	6	12.8	11.5	313	2940	390	830	68.8
	7	14.1	10.4	361	3160	415	780	67.4
	8	14.2	10.2	411	3160	425	710	69.8
E 2% NaCl at 10 cc./min.	1	14.2	0.21	346		395		50.2
	2	11.3	0.23	350		398		48.1
	3	13.5	0.47	493		396		50.2
	4	14.6	1.43	766		422		70.4
	5	16.4	2.69	884		438		66.0
	6	14.2	4.82	804		455		66.8
	7	15.8	6.20	716		465		73.4
	8	14.4	6.08	640		453		72.3

* The glucose infusion was started 2 hours before the beginning of the first period.

Gilman (6) showed that one unit of Pitressin failed to inhibit the diuresis resulting from the injection of 10 per cent saline, while doses $\frac{1}{60}$ as large will sharply depress a water diuresis.

Strong solutions of NaCl may cause the release of the antidiuretic hormone by some direct action on the posterior lobe of the pituitary, or through a reflex originating in some chemoreceptor, or, more probably, through stimulation of the cells of the highly vascular supra-optic nuclei, whose processes pass down the pituitary stalk to the neurohypophysis. Excision of the pituitary or transection of the pituitary stalk with subsequent atrophy of the posterior lobe abolishes the response, but this shows only that the antidiuretic hormone originates in the neurohypophysis, and throws no light on the mechanism of its

release. Sensitivity of the neurones in the supra-optic nuclei to changes in the chemical composition of the blood has been suggested by Craigie (4) and by Ingram, Ladd and Benbow (13), but direct evidence, such as might be obtained by recording action potentials from the nuclei or the pituitary stalk during the infusion of different solutions, has not been provided. Nor have the changes in the blood which cause the pituitary to liberate or withhold the antidiuretic hormone been defined. It may be that rate of change is more important than magnitude, as has been suggested by Baldes and Smirk (3), who investigated the rôle of the osmotic pressure of the plasma in initiating a water diuresis.

MacKay and MacKay (15) and Aitken (1) have lowered the plasma chloride until the urine was chloride-free by urea diuresis, salt-free diets, and sweating. When the lost chloride was replaced, the plasma concentration at which chloride reappeared in the urine was designated the chloride threshold. Aitken's objections to the method, which failed to give reproducible results, led him to recommend that the concept of a chloride threshold be abandoned. In Rehberg's (17) observations on himself, plasma chloride was changed by the ingestion of salt or water, and glomerular filtration measured by creatinine clearances. His calculations of reabsorption of chloride reveal that there is no tubular maximum. However, when the percentage of filtered chloride excreted in the urine was related to creatinine U/P, two curves were obtained representing the results when the plasma chloride was above or below 375 mgm. per cent. This led him to set this plasma concentration as the chloride threshold in man. Of far more interest to us was his finding that after the ingestion of water, chloride was reabsorbed in a concentration higher than that of plasma, while after the ingestion of salt, this relationship was reversed.

In our experiments on dogs, the rate of tubular reabsorption of chloride has been as high as 500 mgm./min., and this upper limit seems to have been imposed by the tolerance of the animal for hypertonic saline, rather than by the inability of the tubules to transfer more chloride back into the blood. Attempts to find a plasma chloride concentration at which significant quantities of chloride began to be excreted were unsuccessful, and determinations of the threshold could not be repeated using the same subject and experimental procedures. When the conditions of the experiments were changed, the threshold varied so widely that it lost all significance. For example, during the glucose diuresis, chloride excretion may proceed at about 50 mgm./min., even though the plasma chloride falls 50 to 75 mgm. below the preinfusion level, and when glomerular filtration is only slightly increased. Since a urea (15) or sucrose (12) diuresis causes a similar chloride excretion at low plasma concentrations, it seems that an osmotic diuresis disturbs tubular reabsorption of chloride. When saline, instead of glucose, was infused, chloride excretion was invariably increased, regardless of the preinfusion plasma chloride concentration or the rate of glomerular filtration. This has been true even when the saline was so dilute (0.2–0.4 per cent NaCl) that the plasma chloride concentration was diminished. On the basis of these data, it is obviously impossible to define a chloride threshold for the dog.

SUMMARY

The renal tubular reabsorption and the urinary excretion of water and of chloride have been studied in normal and diabetes insipidus dogs during water, saline and glucose diureses. The factors which determine the partition of chloride between tubular reabsorbate and urine are not revealed, but it is shown that there is neither a maximal rate of tubular reabsorption nor a renal threshold for chloride. Pitressin does not inhibit its tubular reabsorption nor facilitate its excretion. Renal tubular reabsorption of water is related to the reabsorption of chloride under all conditions of these experiments, and the antidiuretic hormone controls this relationship by regulating water reabsorption.

Hypertonic saline stimulates, while hypotonic saline, glucose solutions, and water inhibit the secretion of antidiuretic hormone. It is proposed that for the regulation of water exchange there is a system which includes the hypothalamico-hypophyseal mechanism, which, through its sensitivity to changes in the salt content of the blood, controls the secretion of pituitrin, and thereby the concentration in which salt is reabsorbed by the renal tubules back into the blood.

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INCREASED RED BLOOD CELL FRAGILITY AFTER FAT INGESTION¹

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There have been indications that ingested fat might be injurious to red blood cells. In studies on relatively few human individuals over brief periods of time, red blood cell destruction apparently proceeded at a faster rate on a high fat diet, as evidenced by an increased urobilin excretion in the feces (Glanzmann, 1929; Salomon, 1920; Josephs, Holt, Tidwell and Kajdi, 1938). The experiments of Freeman, Loewy, Marchello, and Johnson (1942) carried out for many weeks conclusively demonstrated this effect by the more direct method of measurement of the bilirubin excretion in dogs, which was greater with increases of fat in the diet.

Some of the mechanisms involved in this blood destruction from fat ingestion were clarified by Johnson and Freeman (1938), who showed that during the absorption of a fat meal, lymph collected from the lacteals or from the thoracic duct is very hemolytic. It was also demonstrated (Freeman and Johnson, 1940) that fatty acids and soaps, which have presumably escaped resynthesis into neutral fat during absorption, are present in the lymph in quantities sufficient to account for this hemolysis.

Since the lymph entering the blood after a fat meal is hemolytic, more direct evidence of red blood cell injury in the circulating stream at that time was sought. In preliminary experiments on dogs, actual *hemolysis* did not occur when red blood cells were mixed with lipemic serum drawn after a fat meal. The experiments here reported test whether red cells are made more *fragile* by exposure to such serum.

METHODS AND RESULTS. The fragility of red cells mixed with lipemic serum was compared with that of cells similarly mixed with fasting serum. The fragility test involved identical dilutions with distilled water of both sets of blood plus serum mixtures, gentle shaking of these diluted mixtures, and finally, counting the intact cells (per cubic millimeter) in each sample. Addition of the water reduced the inorganic salt concentration to approximately the equivalent of 0.4 per cent NaCl, midway between the concentrations producing beginning and total hemolysis in dogs.

Each dog tested was fed a fat meal consisting of 10 cc. of olive or corn oil per kilogram body weight in addition to commercial canned dog food. Samples of fasting serum ("control fluid") and of lipemic serum (one of the "test fluids") from the same dog drawn at two and at three and one-half hours after a fat meal were each mixed with an equal volume of whole blood (oxalated) from the fasting

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animal. After shaking each mixture for forty-five minutes, distilled water was added and the shaking was continued for thirty minutes. The final mixture contained one volume of red cells (i.e., oxalated blood), one volume of fasting or lipemic serum, and two volumes of distilled water. Ordinary red blood cell counts were then done on each mixture.

To express the results graphically (fig. 1), the red cell count of the control mixture (e.g. fasting serum and oxalated blood) is arbitrarily placed at zero hemolysis; that is, the hemolysis from distilled water and from shaking is disregarded since this is identical in both control and test mixtures. The test

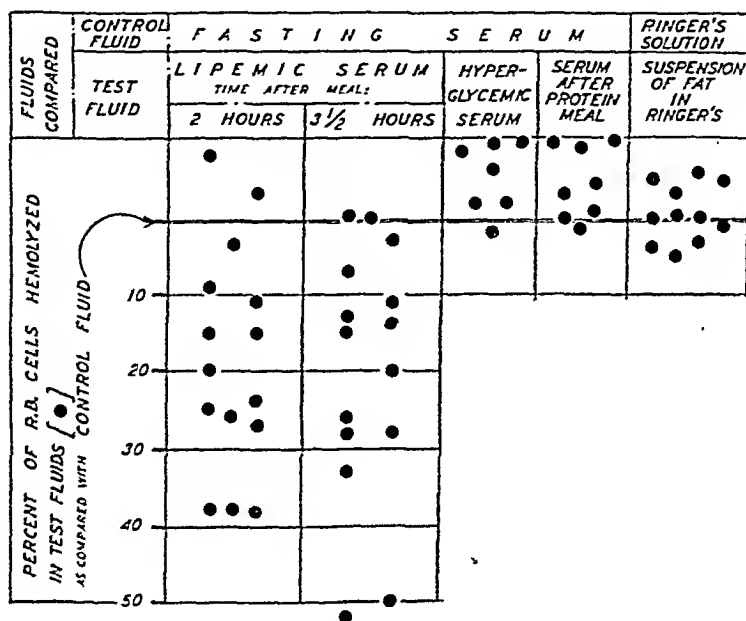


Fig. 1. Change in fragility of red blood cells upon exposure to various test fluids. The test counts are plotted as per cent reduction (the ordinate) from the corresponding control count which is represented by the zero line. To avoid confusion, these control points are omitted.

count is then expressed as percentage reduction from the control. In other words, it represents the increase in hemolysis caused by the test fluid.

When lipemic serum was the test fluid, red cell fragility was significantly increased, as indicated by the lower counts in the test mixtures analyzed statistically. There was no appreciable difference between the effects of the two and the three and one-half hour sera. The average reduction from the control for all lipemic sera tested was 19 per cent.

To check on the possibility that ingestion of other fuel foods might increase red cell fragility, high sugar and high protein meals were fed. The hyperglycemic serum and the serum after the protein meal, respectively, were used as test fluids, substituting for lipemic serum in procedures exactly parallel to those employed with high fat meals. No increase in the fragility of red cells exposed

to these sera was observed. If anything, the *resistance* of the red cells was increased in these caloric controls as indicated by the higher counts in the test mixtures (see third and fourth columns of fig. 1). However, too few experiments have been performed to warrant any final conclusion on this point. Earlier workers have reported that large quantities of sugar do exert a protective effect on red cells preserved *in vitro* (Rous and Turner, 1916). Also, insulin hypoglycemia has been shown to increase the fragility of red cells (Booth, 1941).

Although other studies (Freeman and Johnson, 1940) have shown it to be unlikely that the neutral fat particles in any way affect the fragility of red cells, it was felt worthwhile to test the possibility under the conditions of this experiment. A stable suspension of olive oil in Ringer's solution was used as the test fluid; the particle size and the concentration approximated those in lipemic serum. This was compared with Ringer's solution as the control fluid. Neutral fat was found to be as ineffective as Ringer's solution in altering red cell fragility (see last column of fig. 1). The deviation here was from -6 per cent to $+5$ per cent, perhaps giving a good indication of the limits of error of the method.

DISCUSSION. These experiments demonstrate the presence in dogs' lipemic serum of one or more agents which act directly on red blood cells to increase their fragility. Probably these agents are the free fatty acids and soaps which account for the hemolytic potency of thoracic duct lymph after fat absorption (Freeman and Johnson, 1940). They apparently proceed from the lymph into the blood in sufficient concentration to cause this increase in red cell fragility. Chemical determinations of these substances in the serum after a fat meal have not been done.

The evidence strongly suggests that red cells are injured *in vivo* very soon after the products of fat digestion enter the bloodstream. It follows that the increased blood destruction during a prolonged high fat diet (Freeman, Loewy, Marchello and Johnson, 1942) may be accounted for largely or wholly by continual repetitions of this short-time effect rather than by some longer, more indirect, metabolic process. Indeed the possibility that ketone bodies may be the destructive agents has probably been eliminated in the experiments of Josephs, Holt, Tidwell and Kajdi (1938). Experiments by Freeman, Loewy and Johnson (1943) lend strong support to the concept that free fatty acids or soaps can act almost at once to increase red cell destruction. They demonstrated an increased bilirubin excretion in anesthetized dogs within an hour or two after injection of small quantities of fatty acid or soap into the jugular vein.

It may be, as perhaps suggested by the experiments where sugar and protein were fed, that the non-fat elements of the diet serve in small part to protect against the injurious effect of fat on the red blood cells. However, at least in dogs, fat ingestion probably is an important mechanism in the normal daily red cell destruction.

Considered in the light of this property of ingested fat, it seems probable that the following well-known phenomena have definite adaptive values hitherto not emphasized: 1, the tendency to vomit after a high fat meal; 2, the slower emptying time of the stomach after fat meals than after meals high in carbohydrate or protein; and 3, the absorption of fat into the lymphatic system and its subsequent gradual introduction into the circulating blood. All these prevent the digestion products of ingested fat from entering the blood stream too quickly or in too great amounts. They would appear to be part of a physiological system of checks to prevent excessive red cell destruction. The ultimate check against anemias of increased red cell destruction is the bone marrow which, in animals on prolonged fat diets, is capable of replacing the extra cell losses (Freeman, Loewy, Marchello, and Johnson, 1943). When these checks are inadequate or when the blood cells themselves are more susceptible to fat injury, an anemia may well develop. These possibilities remain to be investigated as causative factors in certain human anemias.

It is of interest that injury from fat ingestion is encountered in cells other than the erythrocytes. A number of recent animal experiments indicate that high fat diets also produce liver damage. These experiments fall into two groups: 1, those in which fat ingestion apparently damages the liver cells directly (Blumberg and McCollum, 1941; György and Goldblatt, 1941); and 2, those in which fat ingestion acts by increasing the susceptibility of the liver to damage by other agents. Deleterious agents which have been used in such experiments are chloroform (Goldschmidt, Vars, Raudin, 1939), carbon tetrachloride (Bollman, 1940) and arsphenamine (Messinger and Hawkins, 1940). In the case of arsphenamine poisoning, when high carbohydrate or protein diets were changed to high fat diets, the icterus index showed a progressive increase. The work presented here suggests that this icterus may be caused in part by increased blood destruction as well as by liver damage. If fat is injurious to both blood cells and liver cells, it would seem to increase the likelihood that fat ingestion may be related to the etiology of certain anemias.

SUMMARY AND CONCLUSIONS

1. After fat ingestion an agent which increases the fragility of red blood cells is present in dog's serum.

2. Neutral fat is not this agent. Previous work indicates that free fatty acids and soaps, reaching the blood stream by way of the lymphatics, are probably responsible for the effect.

3. Ingestion of carbohydrates and proteins does not increase red cell fragility. On the contrary, meals rich in these substances produce serum which may actually protect red cells from water hemolysis.

4. These results, when considered with the finding of increased red cell destruction *in vivo* on a high fat diet, indicate that fat ingestion probably is an important mechanism in the physiological destruction of red cells in dogs.

5. These experiments suggest further that the mechanism of the demonstrated *in vivo* red cell destruction by fat ingestion is immediate and direct and may not involve any relatively long metabolic process.

6. It is suggested that those physiological mechanisms which delay the absorption of fat and its entrance into the bloodstream are probably significant in the prevention of anemia.

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THE EFFECT OF OXYGEN DEPRIVATION ON THE RELATION BETWEEN STIMULUS INTENSITY AND THE LATENCY OF VISUAL AFTER-IMAGES

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The visual mechanism manifests marked changes in function when its oxidative processes are disturbed.¹ Such a disturbance may be caused by exposure to low atmospheric oxygen tensions, as in high altitude flying, and by reducing the supply of metabolites which can be utilized by the central nervous system, as in insulin hypoglycemia (1). During anoxia, the visual field appears to become dim. On readmission of oxygen, it appears to become brighter again. A quantitative and highly sensitive index of such changes in visual sensitivity during anoxia can be obtained by determining the absolute light threshold, as shown by McFarland and Evans (2). They found, for example, that at a simulated altitude of 15,000 feet a light intensity 2.5 times as great as normal was required in order for it to be seen.

Experimental evidence has been presented by both Crozier and Holway (3) and by Hurvich (4) in support of the view that the critical determinants of the visual sensory processes may be central rather than photochemical in nature. The effects of anoxia seem also to be attributable primarily to alterations in the neural elements of the visual mechanism, although the exact locus remains to be determined. The following facts indicate that the photochemical mechanism is not involved: *a.* The light sensitivity of a previously dark-adapted individual is reduced by subsequent oxygen deprivation. It is presumed that the concentration of the photosensitive substances remains unchanged, since no exposure to light has taken place. Furthermore, the decreased sensitivity could not be due to delayed regeneration of these substances, since this process had been completed before exposure to anoxia (5). *b. In vitro*, the rate of decomposition and regeneration of visual purple is not affected by the absence or presence of oxygen (6). *c.* Although different photosensitive substances are concerned with rod and with cone vision, anoxia causes an equal rise in the rod and cone thresholds (7). *d.* The threshold of the eye to electrical stimulation, which presumably does not depend on photochemical mechanisms, also rises during anoxia (8). *e.* On readmission of oxygen, the rate at which sensitivity is recovered is much more rapid than can be accounted for by the rate at which the visual pigments regenerate.

In order to determine the effect of anoxia on a visual function at illuminations of higher than threshold intensities, McFarland and Halperin (9) made a study

¹ For a comprehensive summary of the literature concerning the effects of anoxia on vision, see McFarland, R. A., J. N. Evans and M. H. Halperin. *Arch. Ophthal.* 26: 886, 1941.

of foveal visual acuity in relation to illumination. In normal air, the logarithm of visual acuity rises continuously as the logarithm of illumination intensity is increased—rapidly at first, then more gradually until a maximum is reached and the curve becomes horizontal. During exposure to low oxygen tensions, the form of this curve remains unaltered, but the curve as a whole is translated to the right along the intensity axis. The amount of this translation is of the same order of magnitude, in logarithmic units, as in the case of the absolute thresholds at comparable degrees of anoxia. Studies of other visual functions, such as intensity discrimination (differential sensitivity), have yielded similar results (10).

The relationship between oxygen deprivation and the latency of visual after-images has been investigated in two previous studies. Gellhorn and Spiesman (11) performed experiments on subjects who breathed gas mixtures deficient in oxygen at sea-level pressure. The stimuli were colored squares or a white light, and the fixation time ten seconds. Whereas a reduction of the oxygen concentration to 13 per cent was without effect on the latency of the after-image, changes of considerable magnitude occurred when air containing 9 to 11 per cent oxygen was breathed. Under these conditions, the after-images were either delayed, or they failed to appear at all. In subjects acclimatized to high altitudes during the International High Altitude Expedition to the Chilean Andes, McFarland (12), using a similar method, likewise found a lengthening of the latent period. This prolongation became statistically significant only at 21,100 feet, which corresponds to an oxygen concentration of 9.5 per cent at sea-level pressure.

Certain experimental limitations of these earlier studies, as, for example, their restriction to a single value of the stimulus intensity continuum, suggested the advisability of a more extensive investigation of the behavior of after-images during anoxia. In the present study the intensity of the stimulus was varied over a wide range so that the behavior during anoxia of the entire function might be observed. The present experiments differ from the earlier ones also in employing a brief stimulus duration of 0.2 second. The relationship between stimulus intensity and the latency of after-images has been studied under a wide variety of experimental conditions in normal air (13–17). The results differ widely and depend on such variables as the state of retinal adaptation and the exposure time, size, the retinal location, and spectral composition of the stimulus, as well as the background against which the after-image is observed. The problem becomes complicated when prolonged stimulus exposures are employed, since the eye cannot be maintained motionless for more than a fraction of a second, and adjacent retinal areas are unequally exposed to the stimulus when the eye moves. Also, observation of the after-image against an externally illuminated background results in the appearance of "positive" and "negative" phases of the after-image. Such complicating factors can be minimized by utilizing stimuli of very short duration and observing the after-image in complete darkness. In this manner, fixation is more easily controlled and only "positive" after-images (i.e., images brighter than the background) are seen.

Even under such simplified conditions, the results obtained seem to depend on the intensity range. Hurvich and Joensson (17) made a systematic investigation of the latent time of a bluish-gray after-image which appears approximately 0.5 to 1.5 seconds after cessation of a brief white stimulus. The latent time of this image *decreased* with an increase of stimulus intensity, duration, or area. When the magnitude of any one of these variables was raised beyond certain limits, there was a brilliant after-glow following the cessation of the stimulus, which obscured the appearance of the usual bluish-gray after-image. Feinbloom (16) also used brief stimuli and observed the after-image in complete darkness. He employed, however, a range of stimulus intensities beginning at a higher brightness level than did Hurvich and Joensson. The positive after-image which he observed appeared several seconds after cessation of the stimulus. The latent time of this image *increased* with an increase of stimulus intensity, duration, or area. The after-images described by Hurvich and Joensson differed also in certain qualitative characteristics from those reported by Feinbloom. Whereas the former found the color of the after-image to be bluish-gray regardless of the spectral composition of the stimulus, the latter reported the color of the after-image to vary with the color and intensity of the stimulus.

The present study was performed with the same apparatus and under the same experimental conditions as employed by Hurvich and Joensson (17). The after-image observed under these conditions is described by them as corresponding to that known traditionally as the tertiary or Hess image.

Apparatus. The various components of the optical system employed were mounted on an optical bench and arranged so as to provide independent control of three stimulus variables: (1) intensity, (2) duration and (3) area. In this study only the first of these was varied.

The light source was a 100-watt, 115-volt Mazda projection lamp. A lens served to condense the light rays upon the surface of a milk glass plate (3 x 3 cm.). The latter was placed so that the aperture of the stop delimiting the size of the stimulus field was uniformly illuminated. An aperture of 11.9 mm. diameter, subtending an angle of two degrees at the eye of the observer, was employed. Intensity was controlled by the use of neutral-tint Wratten filters and a specially mounted pair of optical wedges arranged in compensating fashion. The duration of the stimulus exposure was kept constant throughout these experiments at 0.2 second by means of a Compur camera shutter placed between the wedges and an artificial pupil. The latter was 1 mm. in diameter. A synchronizing switch mounted on the shutter made it possible to start an electric timing clock automatically at the end of the stimulation period. The observer's eye was placed at a distance of 35 cm. from the plane of the aperture. A Bausch and Lomb head rest and chin support facilitated the maintenance of fixation. A red fixation dot, 1 mm. in diameter, was continuously visible, being reflected from a glass wafer set at an angle of 45 degrees to the line of regard. The fixation dot was centered in the stimulus field and was also at a distance of 35 cm. from the observer. Extraneous light stimulation was eliminated by placing the observer in a light-proof cubicle painted black.

For the adapting field, a large white matte surface subtending 50 x 65 degrees was fixed on the wall of the chamber adjacent to the apparatus at a distance of 50 cm. from the observer's eye. The intensity of light reflected by this adapting field was kept constant throughout the experiments at approximately ten foot candles.

The measured time interval—i.e., the time elapsing between the cessation of the stimulus and the signal of the occurrence of the after-image—is termed the latent period. To measure this interval, a six-volt battery was connected in series with a precision-timer² reading in hundredths of a second, the synchronizing switch on the camera shutter, and a signal key manipulated by the observer. Before each stimulus presentation, the observer depressed the signal key. At the moment the stimulus field was cut off, the shutter switch was automatically tripped. This completed the circuit and thus started the timer. The latter stopped when the observer signalled the onset of the after-image by breaking the circuit as he released the depressed key.

EXPERIMENTAL PROCEDURE. The experiments were performed in a sealed chamber which permitted the regulation of temperature and ventilation by means of an air-conditioning unit, and the variation and maintenance of the oxygen content of the air at any desired level, the total barometric pressure remaining constant.

The data required for the determination of each curve relating stimulus intensity to latency of the after-image were obtained as follows. Observations were preceded by three minutes of bright adaptation to the ten foot-candle adapting field, followed by five minutes of dark adaptation. All observations were made with the right eye. The measurements were taken in such a sequence as to be homogeneous for intensity. A single measurement was made consecutively at each intensity, passing usually from the lowest to the highest. The direction was then reversed until either three or five readings had been obtained at each point. Following each single determination the observer was given a thirty-second rest period. Additional pauses were introduced at the end of each series (one reading at each of the four or five intensities), and whenever the observer reported fatigue or persistent visual after-effects.

The procedure thus outlined was first carried out in normal air (21 per cent oxygen). Samples of the subject's normal alveolar air were obtained. The concentration of oxygen in the chamber was then reduced to the desired level in about twenty minutes by diluting the air with nitrogen. After about ten minutes' exposure to the low oxygen tension, the experimental procedure was repeated. Samples of the chamber air and of the subject's alveolar air were obtained before and after the visual measurements. They were subsequently analyzed for oxygen and carbon dioxide on a standard Haldane apparatus. The determinations at the simulated high altitude were followed by administration for stated intervals, of 100 per cent oxygen through a mask. The after-image measurements were then repeated ("recovery curve").

² Model S-1, Standard Electric Time Company, Springfield, Mass.

Means and standard deviations (S.D.) were computed for the three or five observations at each stimulus intensity by the usual methods. In those instances in which combined means are given for several experiments (table 1), the combined standard deviation at each intensity was computed according to the equation $S. D. = \sqrt{\frac{\sum p\sigma^2}{n - r}}$, where p is the number of individual observations

TABLE 1

The effect of oxygen deprivation on the latency of visual after-images
(See fig. 1A, B, and C)

SUBJECT	LOG ₁₀ STIMULUS INTENSITY (PHOTONS)	LATENT TIME OF AFTER-IMAGES (SECONDS)*					
		Normal air		Low oxygen†		Recovery with oxygen‡	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
LMH (mean of 3 experiments§ at 10.4-11.2 per cent oxygen)	1.16	1.33	0.08	1.51 (11)*	0.13	1.27	0.20
	1.48	1.17	0.06	1.33	0.13	1.15	0.19
	2.13	1.08	0.07	1.14	0.10	1.10	0.10
	2.79	0.93	0.06	1.02	0.08	0.96	0.06
JDM (mean of 2 experiments at 11.2-11.3 per cent oxygen)	1.20	1.33	0.07	1.53 (3)	0.11	1.35	0.14
	1.52	1.16	0.11	1.30	0.12	1.16	0.15
	2.17	0.87	0.12	0.97	0.21	0.83	0.05
	2.82	0.59	0.07	0.75	0.13	0.67	0.12
MHH (one experiment at 12.5 per cent oxygen)	1.16	1.62	0.37	1.77	0.30	1.60	0.23
	1.48	1.11	0.14	1.45	0.28	1.04	0.26
	2.13	0.85	0.06	0.90	0.03	0.88	0.04
	2.79	0.82	0.10	0.77	0.04	0.72	0.12

* The number of observations represented by each datum is as follows: subject LMH, 13; subject JDM, 8; subject MHH, 5. Numbers in parentheses indicate the number of measurements on which the mean is based in those cases in which the after-image failed to appear during one or more observations.

† See table 4 for the individual alveolar air and chamber air analyses.

‡ The time during which oxygen was breathed subsequent to exposure to the reduced oxygen tension ("recovery time") was as follows: subject LMH, 45-50 minutes; subject JDM, 25-38 minutes; subject MHH, 17 minutes.

§ Recovery curves were obtained in only two of these three experiments. In order to make the mean of these two comparable to the mean of all 3 experiments in normal air, a correction factor was applied. This was derived from the ratio of the means for all three normal-air curves to the means for the normal-air curves in these two experiments.

and σ the standard deviation of these observations in each experiment, n the total number of observations and r the number of experiments.

RESULTS. The relationship between the latency of visual after-images and stimulus intensity,² during exposure to various atmospheric oxygen tensions, is

² Stimulus intensity is expressed in photons (millilamberts $\times \frac{10}{\pi} \times$ pupil area in square millimeters).

shown in table 1 and figure 1 (A, B and C). These data are based on six experimental sessions employing three trained subjects. Each session consisted of a series of measurements in normal air (21 per cent oxygen), followed by repetition of the determinations during anoxia. Finally, 100 per cent oxygen was administered and observations were repeated after stated recovery intervals.

The general form of the function relating latent time to stimulus intensity is curvilinear. The latent time decreases as the stimulus intensity is increased—rapidly at first, then more gradually. Furthermore, a given degree of anoxia causes a delay in the appearance of the after-image over the entire range of stimulus intensities, the magnitude of the delay being a function of the stimulus intensity.

The limited accuracy of the measurements precludes the possibility of a unique mathematical description of the displacement of the curves caused by anoxia—

TABLE 2

The effect of progressive degrees of anoxia on visual after-images

Data from one experiment on subject MHH (see fig. 1D)

LOG ₁₀ STIMULUS INTENSITY (PHOTONS)	LATENT TIME OF AFTER-IMAGE (SECONDS)*									
	Normal air		13.7% oxygen		12.1% oxygen		10.8% oxygen		Recovery with oxygen†	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
0.60	1.38	0.09	2.06	0.44	1.86	0.08	2.19	0.34	1.14	0.05
0.93	1.13	0.14	1.45	0.18	1.53	0.09	1.78	0.04	1.01	0.06
1.56	0.98	0.11	1.11	0.09	1.29	0.21	1.58	0.19	0.91	0.02
2.20	0.78	0.06	0.87	0.06	0.82	0.07	1.10	0.17	0.70	0.04
2.84	0.65	0.06	0.66	0.05	0.72	0.08	0.96‡	0.06	0.68	0.08

* Each entry is the mean of three observations.

† After twenty minutes.

‡ Mean of two observations. After-image failed to appear once.

i.e., whether it is horizontal, vertical, or both. Relatively more precise measurements of visual acuity (9) and intensity discrimination (10) have shown that during anoxia the curves relating these variables to the logarithm of stimulus intensity are displaced solely on the intensity axis. The curves in figure 1 were, therefore, drawn in such a manner as to determine whether the effect of anoxia on the latent time might similarly be described in terms of a horizontal translation along the intensity axis. A curve was first fitted by visual inspection to one set of points in each graph. The same curve was then translated horizontally until it fell on the other set, extrapolating when necessary. In each case, the curve which fits the normal air data was found to fit those for low oxygen as well, when transposed on the intensity axis. The form of the curve is thus identical under all the conditions tested. The effect of anoxia may be described simply as a translation of the curve to the right along the intensity axis, causing a prolongation of the latent time for any given stimulus intensity.

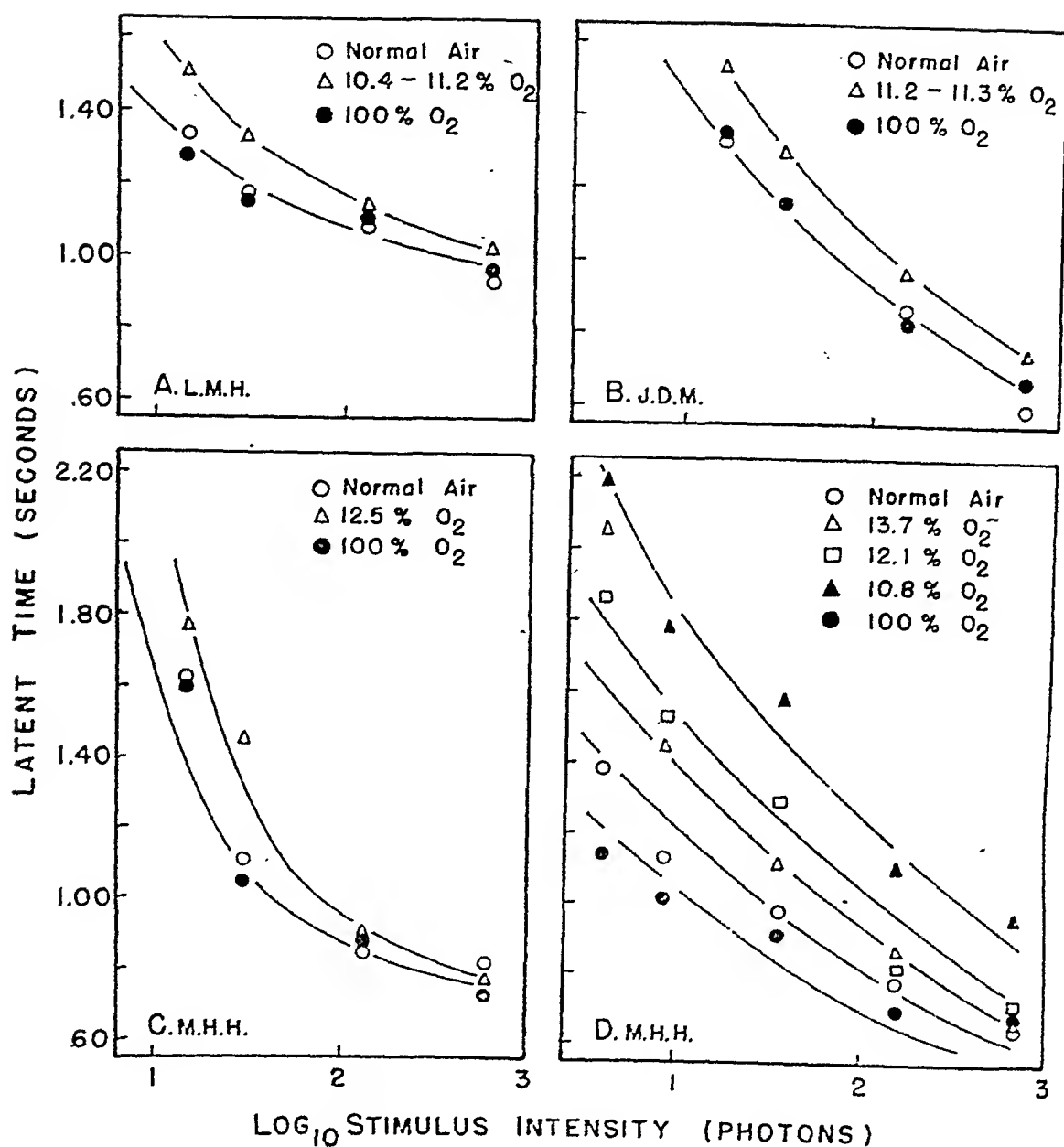


Fig. 1. The effect of anoxia on the relation between the latent time of visual after-images and stimulus intensity. Graphs A, B and C are based on the data in table 1, and graph D on those in table 2. In each case, the observations were made under the conditions and in the sequence indicated by the corresponding legend.

A curve was first fitted to one set of points in each graph and then translated horizontally until it fell on the other set or sets of points extrapolating when necessary. (In graphs A, B and C, the open and solid circles were treated as a single set.) Since in each graph a satisfactory fit to each set of points could be obtained by horizontal translation of the same curve, the effect of anoxia may be described as a displacement of the curve to the right on the intensity axis. This results in a prolongation of the latent time for any given stimulus intensity.

This translation is similar in direction and magnitude (when expressed in logarithmic units of intensity) to that observed in the case of foveal visual acuity (9) and intensity discrimination thresholds (10), during comparable degrees of anoxia. The meaning of such a translation in each of the three cases is that a greater intensity of light is necessary during anoxia to produce a given visual response. This is equivalent to the effect of a reduction in the intensity of the stimulus by placing a light filter or smoked glass before the observer's eye. At low intensities of illumination, where the curves are steepest and a small decrease in illumination causes a large change in the visual function, anoxia similarly causes a large change. At progressively higher intensities of illumination, where the curves are less steep and a proportionate decrease in illumination causes less change in the visual function, anoxia likewise has a progressively smaller effect. This dependency of the magnitude of change in latent time during anoxia on stimulus intensity is confirmed by the data in table 1. These indicate that the prolongation of latent time is greater for stimuli of low intensity than for more intense ones. In fact, the least intense stimulus employed, which always yielded an after-image in normal air, frequently failed to produce one during oxygen deprivation, the latency thus being prolonged indefinitely. For example, in the case of subject JDM, during anoxia an after-image appeared in only three of a total of eight trials at the lowest intensity.

The effect of successive exposure to three progressively more severe degrees of oxygen lack is shown in figure 1 (D) and table 2, which present the data for one subject. A curve was fitted to one set of points by visual inspection and translated horizontally to fit the other sets. In general, the extent to which the curve is displaced varies directly with the degree of oxygen deprivation. There is, however, some overlapping of the data at the first two stages of oxygen lack. This experiment, in which only three observations were made at each point, required approximately five hours. Because of the small number of observations which were thus possible, conclusions as to the quantitative relation between the degree of oxygen deprivation and the change in the latency of the after-images cannot be drawn. Furthermore, control experiments on other subjects, who made an equal number of observations over a period of four hours in normal air, also showed a tendency toward a lengthening of the latent time toward the end of the experiment, possibly as a result of fatigue. It is possible, therefore, that a part of the change at the lowest oxygen concentration may also be attributable to fatigue. The complete reversal of the change upon administration of oxygen suggests, however, that the prolongation was due primarily to anoxia. Here, again, the changes were greatest at the lowest intensities (where the curve is steepest) and least at the brightest intensities (where the curve is less steep).

Experiments on light sensitivity (2) and on foveal visual acuity (9) have indicated that when oxygen is administered subsequent to oxygen deprivation full recovery of these functions takes place in three or four minutes. It was anticipated, therefore, that the latent time of the after-images would behave in a similar fashion. Preliminary experimentation indicated very clearly, however, that three or four minutes of oxygen inhalation failed to produce even an appre-

cial degree of recovery. As table 3 and figure 2 indicate, the curve relating latency to stimulus intensity does not return to its original position even after a

TABLE 3

Slow recovery of latent time of after-image subsequent to anoxia

Data from one experiment on each of two subjects (see fig. 2)

SUBJECT	LOG ₁₀ STIMULUS INTENSITY (PHOTONS)	LATENT TIME OF AFTER-IMAGE (SECONDS)*							
		Normal air		10.4% oxygen		Recovery with oxygen—10 min.		Recovery with oxygen —50 min.	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
MHH	0.85	1.97	0.22	2.34	0.35	1.99	0.21	1.91	0.20
	1.16	1.38	0.30	1.80	0.09	1.50	0.21	1.37	0.16
	1.48	1.10	0.08	1.42	0.15	1.31	0.19	1.12	0.15
	2.13	0.89	0.10	0.94	0.09	0.91	0.10	0.87	0.09
	2.79	0.69	0.13	0.89	0.16	0.75	0.06	0.63	0.07
LMH	1.16	1.31	0.07	1.65	0.16	1.41	0.13	1.32	0.13
	1.48	1.25	0.06	1.38	0.08	1.37	0.12	1.21	0.09
	2.13	1.06	0.06	1.16	0.08	1.17	0.06	1.16	0.09
	2.79	0.97	0.03	1.10	0.09	1.02	0.06	0.96	0.04

* Each entry is the mean of 5 observations.

TABLE 4

The mean oxygen and carbon dioxide content of the alveolar air and of the chamber air during the experiments reported in tables 1, 2 and 3

	SUBJECT	ALVEOLAR AIR				CHAMBER AIR		
		Normal		In low oxygen		CO ₂	O ₂	Equivalent altitude
		CO ₂	O ₂	CO ₂	O ₂			
		mm. Hg	mm. Hg	mm. Hg	mm. Hg	per cent	per cent	feet
Table 1	LMH*	38	100	32	40	0.22	10.81	17,000
	JDM†	38	103	35	41	0.29	11.24	16,000
	MHH	39	99	34	50	0.20	12.52	13,300
Table 2	MHH‡	39	99					
	1st alt.			37	57	0.21	13.66	11,000
	2nd alt.			36	46	0.23	12.14	14,000
	3rd alt.			32	31	0.50	10.89	17,000
Table 3	MHH	42	95	34	35	0.18	10.51	17,600
	LMH	38	97	32	35	0.18	10.36	18,000

* Mean of 3 experiments.

† Mean of 2 experiments.

‡ Three successive "altitudes" in single experimental session.

ten-minute recovery period. It should also be noted that oxygen was still being breathed during these observations which lasted twenty minutes. After oxygen had been breathed for fifty minutes, the measurements were repeated and com-

plete recovery was found to have taken place. Apparently, the severity of anoxia is a factor in determining the recovery time. In an experiment on subject MHH (table 1, fig. 1 (C)) a seventeen-minute recovery period sufficed after exposure to 12.5 per cent oxygen whereas a longer period was necessary after exposure to 10.5 per cent oxygen (fig. 2 (A)). Similar findings in relation to

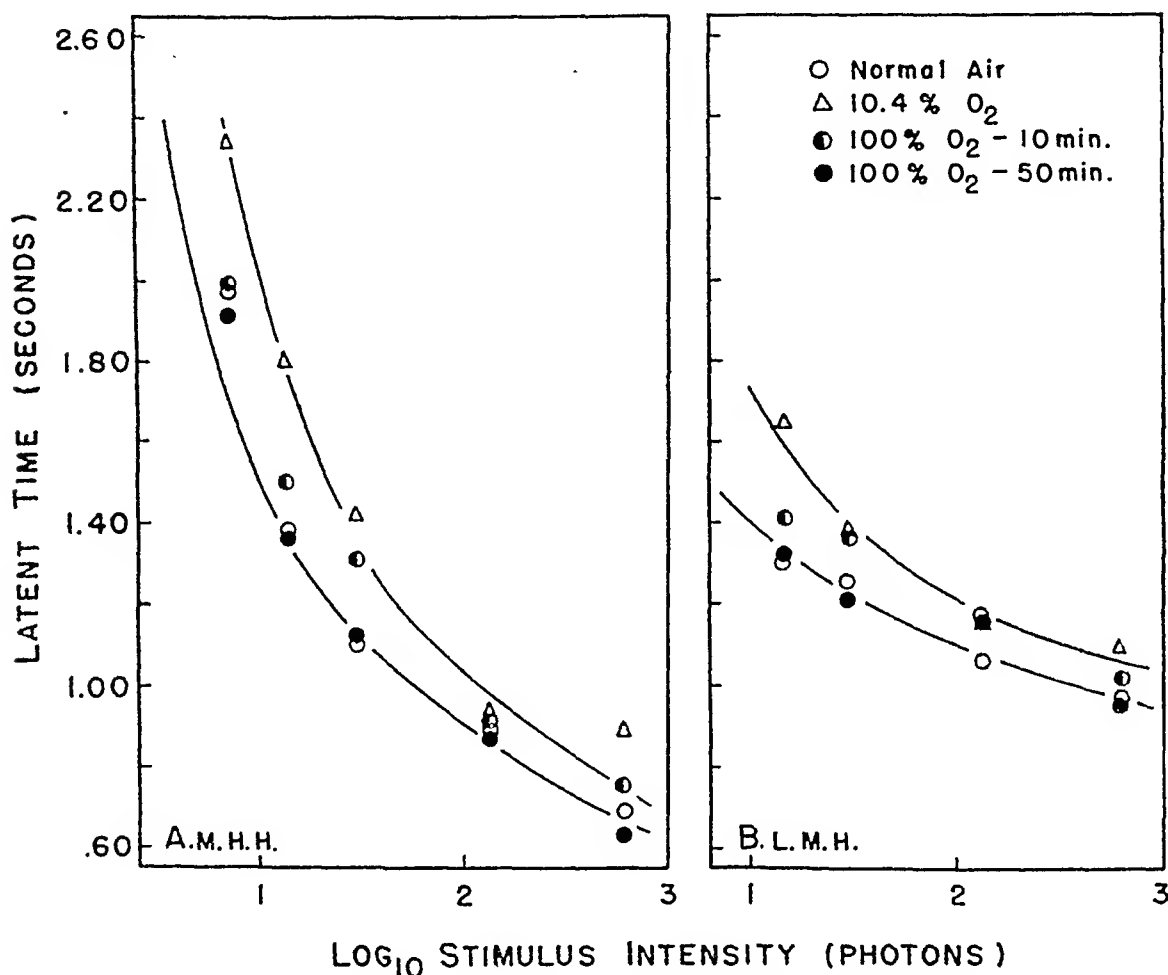


Fig. 2. The slow recovery of the latent time subsequent to anoxia. The half-solid circles, representing measurements begun after oxygen had been breathed for ten minutes, indicate that only partial recovery had taken place. After fifty minutes with oxygen, recovery was complete. This may be contrasted with the rapid recovery of light sensitivity, which is complete within several minutes when oxygen is administered after oxygen-deprivation.

The two curves in each graph are identical, a curve having been fitted to one set of points and translated horizontally to fall on the other set. The open and solid circles were treated as a single set.

recovery from anoxia have been reported by Gellhorn and Spiesman (11), who observed that in some cases the latent period remained lengthened ten minutes after exposure to atmospheres deficient in oxygen.

The results of the analyses of the alveolar air and chamber air samples obtained during the experiments described in tables 1, 2, and 3 are presented in table 4. The findings are consistent with those of earlier experiments.

DISCUSSION. The measure of latent time employed in these experiments is of necessity a composite one, involving not only the true latent time of the visual function, but also the reaction time of the observer. The question arises, therefore, as to how much of the observed change during oxygen deprivation is due to each of these components. Earlier experiments on simple reaction time (18) have shown a prolongation of only about 0.02 second under conditions of anoxia even more severe than those in this experiment. The changes observed in the present study are of a much greater order of magnitude. Consequently, by far the largest proportion of the observed difference is due to an alteration in the true latent time. Furthermore, the fact that after-images frequently failed to appear at all in response to weak stimuli during anoxia indicates a change in the sensory rather than the motor mechanism involved in the subject's response. Prolongation of the reaction time would be expected to result in an upward translation of the curve on the time axis. It is not possible to ascertain from the data obtained in this experiment whether the translation of the curve during anoxia has a vertical component in addition to the horizontal one.

An analysis of the mechanisms underlying the change in latency of the after-image during anoxia cannot be presented, since the mechanisms responsible for the after-images in normal air are unknown. In a general way the relation between the latency of the after-image studied in this experiment and stimulus intensity resembles that between the latency of the electrical after-discharge observed in studies of the optic nerve potential and stimulus intensity (17, 19). The latent time of each of these phenomena decreases as the stimulus intensity is increased. The order of magnitude of the latent time of the after-image is, however, much greater than that of the electrical after-discharge in the organisms studied to date. No reports have been published which deal with the optic nerve potentials during oxygen lack. Further correlation of these phenomena is, therefore, not possible at the present time.

The fact that the action of anoxia on the latency of after-images is comparable to decreasing the brightness of the stimulus (the latent time being prolonged in both cases) suggests the possibility that the prolonged latency may be closely associated with the apparent dimming of the stimulus which is known to take place during oxygen deprivation. However, when oxygen is administered after exposure to low oxygen tensions, the visual field rapidly becomes brighter; light thresholds return to normal levels within three to four minutes. Yet the latency shows practically no recovery after one breathes oxygen for a similar length of time. Consequently, other factors than those causing the change in apparent brightness of the stimulus must be involved and are probably related to a change in the velocity of the physiological processes underlying the eventuation of the after-image. Additional evidence that the changes in some visual functions during anoxia are not due solely to the factors producing the change in apparent brightness of the stimulus has been brought forward by Berger, McFarland, Halperin and Niven (20). They studied the effect of oxygen lack on the resolving power of the eye, employing as a test object two luminous points on a dark ground. The resolving power, as measured by this method and in the intensity

range employed, improves when the brightness of the test points is decreased. It was expected, therefore, that anoxia, which is associated with an apparent dimming of the test points, would also improve the resolving power. On the contrary, an impairment took place, indicating the operation of other factors.

The practical importance of after-images in relation to anoxia is probably not as great as that of visual acuity or light-sensitivity. The slowness of the recovery of this function when oxygen is breathed after exposure to low oxygen tensions may, however, be of practical significance. The behavior of the visual mechanism is believed to reflect changes in the central nervous system, since the retina is embryologically a part of the central nervous system and resembles it metabolically and anatomically. The slowness of the recovery of the function studied here may, therefore, be indicative of a similar delay in the recovery of other central nervous functions. This emphasizes the importance of the use of oxygen by airplane personnel at high altitudes as a preventive measure before any appreciable effects of anoxia occur. If, instead, oxygen is used only after the changes have already taken place, a comparatively long time may be required to reverse them. The possibility that some central nervous functions recover slowly after anoxia suggests, furthermore, that certain factors contributing to landing accidents at sea-level after high altitude flights without an adequate oxygen supply may be attributable to the residual effects of oxygen lack.

SUMMARY

1. The effect of oxygen deficiency on the latent time of the "tertiary" visual after-image was measured in a low oxygen chamber over a wide range of stimulus intensities. Nine experiments were performed on three trained observers.

2. In normal air, there is an inverse relationship between the latent time of the after-image and the intensity of the stimulus; a reduction of the intensity of the stimulus prolongs the latency of the after-image.

3. Anoxia prolongs the latency of the after-image. This effect is consistent with the apparent dimming of the visual field which occurs during oxygen deprivation.

4. The magnitude of the increase in the latent time during anoxia is inversely related to the intensity of the stimulus.

5. During oxygen deprivation, the curve relating the latent time of the after-image to the logarithm of stimulus intensity is translated horizontally to the right on the intensity axis. This displacement is in the same direction and of the same order of magnitude as in the case of the curves relating foveal visual acuity and intensity discrimination to the logarithm of stimulus intensity. The data are not sufficiently precise to rule out the possibility of a slight vertical translation as well.

6. The complete recovery of the latent time upon administration of oxygen may require up to fifty minutes, depending on the degree and duration of the antecedent oxygen deprivation and other factors. The apparent brightness of the visual field, and light thresholds, is restored to normal within three to four minutes. Consequently, the factors underlying the apparent dimming of the

stimuli cannot be the only ones responsible for the prolongation of the latent time of the after-image.

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THE EFFECT OF CYANIDE AND OTHER METAL BINDING SUBSTANCES ON THE PHARMACOLOGICAL ACTION OF EPINEPHRINE¹

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In the course of studies on the mechanism of secretion of the intraocular fluid, it has been shown that the secretory activity of the ciliary body is intimately connected with the existence in the tissue of a chain of redox reactions which link the cytochrome oxidase systems of the epithelium with the dehydrogenase enzymes and substrates of the stroma (1). This redox chain can be activated by the presence of minute amounts of epinephrine in the tissue (2). These experiments have not revealed whether some derivative of epinephrine enters directly as a mediating link in the redox chain, or whether the activation occurs in some more indirect manner. However, the first of these alternatives is at least possible for it has been shown by direct enzymatic studies that a derivative of the oxidation of epinephrine by the cytochrome system or by polyphenol oxidase can act as an oxidative mediator (3).

In view of these findings it was natural to inquire whether the pharmacological activity of epinephrine² on other adrenergic organs might occur through the activation by epinephrine of some redox pathways in the tissue metabolism which are not utilized by the tissue in its resting state. This large question regarding the mechanism of pharmacological action of epinephrine, we have not so far been able to subject to direct experimental test. A preliminary approach to the problem, however, is furnished by the following argument. The oxidative chains which we are considering require as their first step the oxidation of epinephrine by an iron or copper containing system. Such a system would be expected to be cyanide sensitive. The degree of cyanide sensitivity of the adrenergic reaction of various smooth muscle preparations is readily susceptible to experimental investigation. The present report concerns experiments undertaken with this end in view.

The muscle of the rabbit's small intestine is a particularly favorable organ for these experiments since, when suspended in glucose-Tyrode solution, this tissue undergoes continuous rhythmic contractions. The administration of epinephrine causes an arrest of these contractions. On such an organ it is readily possible, therefore, to distinguish the effect of a small dose of cyanide which inhibits the epinephrine effect from that of a larger dose of cyanide which abolishes the rhythmic contraction of the muscle altogether. Most of our experiments were, therefore, performed on this organ. It is possible, however, to extend these studies also to organs in which epinephrine excites muscular contractions.

¹ This work was supported in part by the John and Mary Markle Foundation.

² The epinephrine for our experiments was kindly supplied to us by Parke, Davis & Co.

When, in such an organ, the response to epinephrine has been abolished by cyanide, it is still possible to distinguish between an inhibition of the adrenergic response and a paralysis of the muscle by testing the response to some other pharmacodynamic agent. Experiments of this type will also be reported below.

Scattered and somewhat conflicting reports are to be found of experiments similar to those here contemplated. Evans (4) found that the blood pressure response to epinephrine was depressed in cyanide poisoning though the vessels still responded to pituitrin and barium. However, he reports synergism between cyanide and epinephrine on the intestine. Masing (5) found that cyanide inhibited contraction of the hepatic vessels by epinephrine but found that the glycogenolytic effect of epinephrine was not inhibited by cyanide. This latter is in agreement with Cori's conclusions that the glycogenolytic effect of epinephrine involves essentially anaerobic mechanisms (6). Hazama (7) reported that cyanide in small doses abolishes the inhibitory effect of epinephrine on the intestine and itself caused slight excitation of this organ and Starkenstein (8) found that cyanide excited contractions in the epinephrine inhibited intestine.

Our own experiments were performed exclusively on excised organs suspended in Tyrode solution, thus avoiding the possibility of complex and indirect effects that may be present in experiments on the intact animal. However, experiments on such isolated tissues are also not devoid of their own special complications. In particular we found it necessary early in our work to attempt to distinguish between effects taking place in the bathing fluid and those taking place within the tissue. It is well known that epinephrine undergoes oxidation when placed in Tyrode solution and rapidly loses its pharmacological potency. The suppression of heavy metal catalysis of such oxidation by cyanide would lead to a larger effective dose of epinephrine reaching the tissue when cyanide is present in the bathing fluid than when cyanide is absent. In order to avoid the confusion of true pharmacological effects by chemical reactions occurring in the bathing fluid it was, therefore, necessary for us to find some way of controlling the oxidation of epinephrine in the bathing fluid.

Bathing fluid effects. The literature on "auto-oxidation" of epinephrine is quite extensive and has recently been reviewed by Starkenstein (8), and by Clark and Raventós (9). A great variety of substances have been found to inhibit the auto-oxidation of epinephrine in vitro, and several of these have been shown to enhance and prolong the response to epinephrine of various tissue preparations. Among the factors which enhance the rate of epinephrine auto-oxidation are: high pH, traces of iron or copper, exposure to glass surfaces. In Tyrode solution, the rate of auto-oxidation of epinephrine is 5 times that in blood.

Among the substances which diminish the auto-oxidation of epinephrine are pyrogallol, ascorbic acid, glutathione, amino acids, serum and tissue extracts. It is likely that what has been called "sensitization to epinephrine" by serum in the pharmacological assay technique (Schlossmann and others) (10) is due to prevention of auto-oxidation. The same holds true for sodium citrate which is recommended as a sensitizer in epinephrine assay by Stuber (11).

Our experiments were performed on isolated rabbit's small intestine and on non-pregnant rabbit's uterus using the arrangement of Magnus (12).

The test tissue was suspended in 100 cc. well aerated Tyrode solution containing 0.1 per cent dextrose. The ratio of bathing fluid volume to tissue volume is important in these experiments. If the fluid volume is greatly reduced, for instance to 10 cc. for 1 gram of tissue, the bathing fluid effects reported below are much less striking. The temperature was kept between 38° and 40°C. by a water bath. The pH of the bathing fluid was approximately 8.5. The animals from which the tissue was taken were either anesthetized with nembutal or killed by intravenous air injection or by a blow on the neck. The intestine preparations (usually ileum) were used on the day of their removal. Uterus preparations were used either fresh or after storage in the refrigerator for a day or two.

In the search for a satisfactory method of controlling auto-oxidation of epinephrine in the bathing fluid, various substances were added to the Tyrode solution immediately before the addition of epinephrine. In table 1 are listed some substances which may variously be thought of as antioxidants or as metal binders which we have found to enhance the reactivity of the tissue to epinephrine. All these substances have the same effect (fig. 1). In the dosage used,

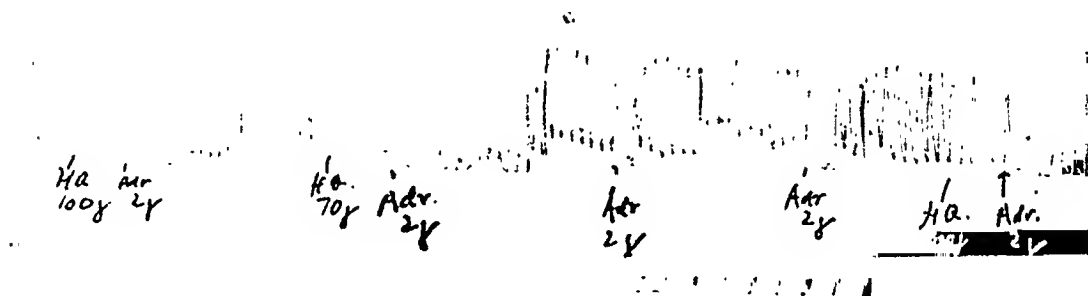


Fig. 1. Effect of the presence of S-hydroxyquinoline (HQ) in the bathing fluid on the response to epinephrine (Adr.). Rabbit's intestine.

none of them caused any pharmacodynamic reaction of the muscle preparation, but the reaction produced by epinephrine added to the Tyrode solution shortly after any of these substances was much more intense and much more prolonged than that produced by the same dose of epinephrine when these substances were absent.

Attention is particularly directed toward those colloids—lens proteins, egg albumin, gelatin, gum arabic—which when added to the Tyrode solution produced the same effect as the antioxidants and metal binders. These colloids were thoroughly dialyzed against distilled water before use in these experiments and their effects constitute conclusive evidence that the phenomenon observed concerns reactions in the bathing fluid, not in the tissue. The protecting substances must be added to the Tyrode solution at least a few seconds before the epinephrine. If added simultaneously with the epinephrine, even if mixed with the epinephrine before addition, their effect is small or absent. It follows that the primary reaction of these protecting agents is with some substance in the Tyrode solution (probably copper) not with epinephrine. Lens protein and egg albumin cause the Tyrode solution to foam badly and were, therefore, not used

in subsequent experiments. Gelatin, if allowed to stand for a long time in well aerated Tyrode solution, finally loses its protective value—possibly through oxidation of its sulphydryl groups. In the experiments to be reported below, gelatin was added to the bathing fluid only shortly before epinephrine. Gum arabic, on the other hand, keeps its protective capacity for a long time and may be added in the stock Tyrode solution.

Effects of cyanide. The effect of cyanide on the epinephrine response was first tested on the rabbit's intestine. In order to distinguish between reactions of cyanide in the bathing fluid and genuine pharmacological effects, the cyanide experiments were performed both with and without the addition of gelatin or gum arabic to the bathing fluid. In general, the cyanide was added one-half to one minute after the gelatin or gum arabic and the same interval before the epinephrine. If no gelatin or gum arabic is used, three different effects of cyanide can be observed. In very low concentration² (about 2.10^{-6} molar) cyanide abolishes or diminishes the effect of a small dose of epinephrine given subse-

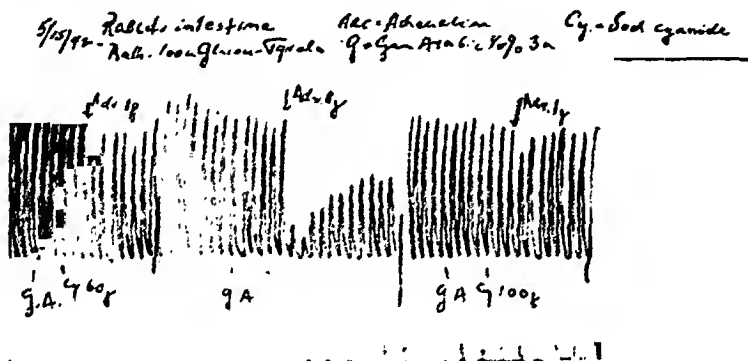


Fig. 2. Effect of medium concentrations of cyanide on the response to epinephrine in the presence of gum arabic in the bathing fluid. Intestine.

quently. In somewhat higher concentration (about 10^{-5} molar) cyanide enhances and prolongs the effect of epinephrine. In still higher concentrations (about 6.10^{-5} molar) cyanide abolishes the normal peristalsis of the tissues. The effect of the medium concentrations of cyanide resembles that of protective antioxidants or metal binders in the bathing fluid. That this is a correct interpretation is shown by experiments with gelatin or gum arabic in the bathing fluid. With these protecting substances present, only two effects of cyanide can be distinguished. With low and medium doses of cyanide, the reaction to epinephrine is diminished or inhibited (fig. 2). With high doses of cyanide, the muscle is paralyzed.

The inhibition of the adrenergic response produced by cyanide is complete only for very small doses of epinephrine. If larger doses of epinephrine are used, the inhibition of cyanide is overcome. The inhibition may be competitive but quantitative relations could not be worked out since it was impossible to use large

² The concentration levels at which these effects were obtained varied considerably in different tissue preparations.

doses of cyanide to balance large doses of epinephrine on account of the resulting paralysis of the muscle. The antagonism between epinephrine and cyanide can be exhibited also if cyanide is added after the epinephrine, in which case the adrenergic response is rapidly terminated.

Precisely similar results were obtained on the uterus preparation (fig. 3) except that, since the response of this organ to epinephrine consists in a contraction of the muscle, the suppression of the adrenergic response by small doses of cyanide could not be immediately distinguished from a paralysis of the muscle produced by high doses of cyanide. This distinction could, however, be made by stimulating the muscle either with pituitrin or with larger doses of epinephrine.

Other substances which have a cyanide-like effect. In order to throw light on the mode of action of cyanide in inhibiting adrenergic reactions, we have sought for other substances which might have a similar effect.

Several of the substances listed by Stotz, Harrer and King (13) and by Graubard (14) as copper binders were tested. A number of these gave excellent bath-

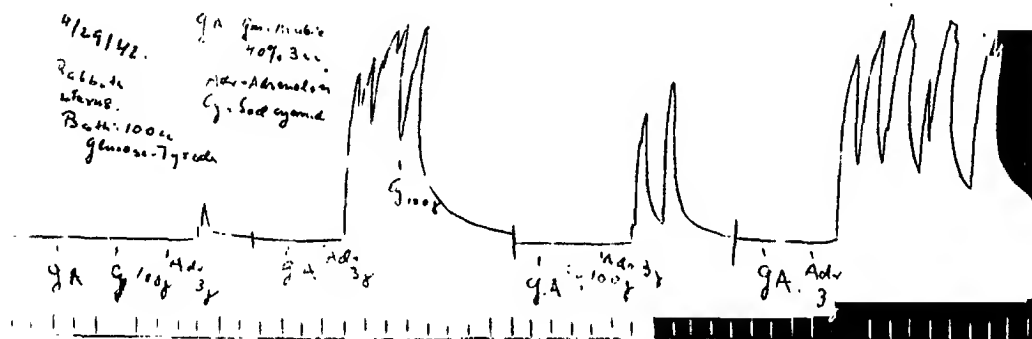


Fig. 3. Effect of medium concentrations of cyanide on the response to epinephrine in the presence of gum arabic in the bathing fluid. Uterus.

ing fluid protection and are listed in table 1. The results with these substances—xanthate, dithiocarbamate, hydroxy-quinoline—suggest that the epinephrine destroying catalyst in the bathing fluid may be copper. Two substances among the “copper binders” showed effects similar to cyanide. These were thiourea and allyl thiourea in concentrations of about 10^{-3} molar and 10^{-4} to 10^{-3} molar resp. In the presence of gelatin or gum arabic these substances inhibit or diminish the adrenergic response both of the intestine and of the uterus. As in the case of cyanide, the inhibition could be overcome by larger doses of epinephrine. The toxic effects of larger doses of the thioureas were not investigated. In the absence of gelatin or gum arabic, threshold doses of the thioureas inhibited the adrenergic reaction. Larger doses yielded enhancement of the adrenergic reaction attributable to a bathing fluid effect. In this respect also these substances closely resemble cyanide.

DISCUSSION. The biochemical effects of cyanide have generally been classed as falling into three types of primary reactions: metal binding, ketone binding, reduction. Thiourea can bind heavy metals, but does not react readily with

ketones and is at best a feeble and sluggish reducing agent. Since the same effect was achieved by cyanide and by thiourea, the implication is strong that the effect was produced by the inactivation of a heavy metal catalyst. Since, in all probability, thiourea does not strongly bind iron it can be suggested that the heavy metal concerned is probably not iron but may be copper. We can conclude, therefore, that the adrenergic reaction of the smooth muscles studied requires for its operation the presence in the tissue of a heavy metal component. These experiments, of course, do not imply that the reaction catalyzed by the heavy metal must be an oxidative one nor that epinephrine itself is the substrate oxidized, though neither of these suggestions is incompatible with the results of these experiments.

TABLE 1

Substances which enhance and prolong the epinephrine response of the rabbit's intestine

CRYSTALLOIDS	CONCENTRATION	COLLOIDS	CONCENTRATION
Sodium diethyldithiocarbamate.....	1.10^{-6}	Dialysed lens proteins	?
Potassium ethylxanthate.....	1.10^{-5}	Egg albumin	1.10^{-3}
8-Hydroxy-quinoline.....	5.10^{-7}	Dialysed gelatine	1.10^{-4}
Sodium citrate.....	1.10^{-5}	Dialysed gum arabic	1.10^{-2}
Ascorbic acid.....	1.10^{-7}		
Glutathione.....	1.10^{-7}		
Tryptophane.....	3.10^{-4}		
Tyrosin.....	$\frac{1}{10}$ sat.		
Adrenalone (keto-compound to epinephrine*)	5.10^{-7}		
1(3-4-dihydroxyphenyl-) 2 aminopropanone (keto-compound to corbasil)*.....	5.10^{-7}		

* These two substances were kindly furnished us by the Winthrop Chemical Company.

SUMMARY

Under appropriate conditions cyanide, thiourea, and allyl thiourea can be shown to inhibit or interrupt the adrenergic response of certain smooth muscle preparations. The anti-adrenergic effect occurs with concentrations of these substances which leave unchanged the capacity of the muscle to respond to non-adrenergic stimuli. These phenomena have been demonstrated both on the rabbit's non-pregnant uterus in which the epinephrine normally excites contractions, and on the rabbit's small intestine in which epinephrine normally inhibits contractions.

It is concluded that the adrenergic response in these organs requires for its operation the presence in the tissue of a heavy metal component, possibly copper.

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SOME EFFECTS OF LOW BAROMETRIC PRESSURES ON KIDNEY FUNCTION IN THE WHITE RAT¹

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In a recent monograph on the effect of anoxia on the body, Van Liere (1942) wrote of oxygen lack and the secretion of urine: "Since, however, anoxic anoxia at times may produce an oliguria, and at other times a polyuria, it is quite clear that more work is needed to establish clearly the mode of action of anoxia on the kidney."

Experiments reported by Van Liere and co-workers (1935) on barbitalized dogs breathing oxygen tensions from 5 to 12 per cent oxygen (equivalent to altitudes from 14,000 to 30,000 ft.) and by Toth (1937) on dogs anesthetized with dial and breathing oxygen tensions of approximately 8 per cent (equivalent to 24,000-25,000 ft.) agreed in that the majority of such animals responded to low oxygen tensions by oliguria; but occasional animals, in contrast, developed marked polyuria. Van Liere suggested that the oliguric threshold was approximately 12 per cent oxygen (about 15,000 ft.), below which the urine output was decreased.

The use of anesthetics undoubtedly influenced the above results, for in later experiments Toth (1940) reported that in unanesthetized dogs with bladder fistulae polyuria was the common response to low oxygen tension. Langley and Clarke (1942) maintained unanesthetized dogs in a low-pressure chamber at 20,000 feet altitude equivalent, and observed a polyuria on the first day which tapered off on the following days of the experiment.

Species difference appears to be as important as anesthetics in producing discordant results, for Armstrong (1939) has shown that humans subjected for 4 or 7 hours daily to a simulated altitude of 12,000 feet (483 mm. Hg) in a low-pressure chamber developed a polyuria in which the urine output was increased from 100 to 300 per cent over normal. McFarland and Edwards (1937) had previously reported that men making transatlantic flights at altitudes of 8,000 to 12,000 feet showed no urinary volume changes in the case of two observers, but a tendency toward polyuria in the airmen responsible for the handling of the ship. These conditions could not be considered controlled, however, and emotional and other influences undoubtedly were operative.

Experiments performed in this laboratory over the past 3 years on unanesthetized white rats in a low-pressure chamber have indicated that these animals respond to high altitudes in a manner corresponding to human beings rather than to dogs. Thus, while 3-hour exposure to 10,000 feet altitude equivalent pro-

¹ This investigation has been made with the assistance of grants from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association, and from the Ella Sachs Plotz Foundation.

duced no change in their urine output, exposure to 15,000 feet invariably resulted in polyuria, which, moreover, did not decrease or disappear on repeated exposure (Silvette, 1942).

Other experiments on renal function in unanesthetized white rats in low-pressure chambers will be reported below. The conditions of these experiments were chosen to simulate actual flight conditions as closely as possible. The metabolic period was 3 hours in every case, and experiments were designed to illustrate the effects of discontinuous (repeated daily) exposure rather than continuous exposure. Long or continued exposure to high altitudes without additional oxygen supply represents an entirely different problem from that investigated here.

Our experiments may conveniently be divided into the following groups:

1. Effect of various high altitudes on urinary output.
2. Influence of temperature on urine and phenolsulphonphthalein excretion at high altitudes.
3. Effect of anemic anoxia on urine output.
4. Influence of preliminary water-load on urinary excretion at high altitudes.
5. Effect of repeated daily exposure to high altitudes on urinary excretion and kidney weight.
6. Effect of post-pituitary extract on the polyuria of high altitudes.

APPARATUS. Two types of low-pressure chambers were constructed, both of which were fitted with automatic devices for maintaining both constant temperature and a constant low pressure. The first was designed to hold 12 individual urine metabolism cages, but because of the reinforced wooden structure of this chamber only experiments up to 15,000 feet altitude equivalent (428 mm. Hg) were performed therein. For experiments from 15,000 feet to 30,000 feet altitude equivalent (up to 226 mm. Hg), four glass desiccators, each of 9 liters capacity, were enclosed in a miniature constant temperature room, and 3 animals placed in a divided cage in each low-pressure (desiccator) chamber. Urine was collected under paraffin oil, individually in the case of experiments up to 15,000 feet, and pooled in four groups of 3 rats in experiments at higher altitude equivalents. A continuous measured supply of fresh air was permitted to pass through the low-pressure chambers, the rate of flow being at least ten times the calculated requirements of the 12 animals comprising each single experimental group.

RESULTS. 1. *Effect of various high altitudes on urinary output.* The average urinary output of 84 control white rats while in the low-pressure chamber but at room pressure and temperature was 0.4 cc. per 100 grams body weight per 3-hour metabolism period. The urine output at 10,000 feet altitude equivalent (523 mm. Hg) was not significantly different from the control figure, but at 15,000 feet altitude equivalent (428 mm. Hg) the average urine output rose to 1.0 cc. urine per 100 grams body weight during the metabolic period, an increase over the normal of 150 per cent. This polyuria was still more marked at an altitude equivalent of 25,000 feet (282 mm. Hg), for in 63 rats maintained at this pressure for 3 hours the average urine output was 1.6 cc. per 100 grams, or an increase of 300 per cent over the control levels.

The high-altitude polyuria could apparently be modified to a certain extent by previous exposure to lower altitudes. Figure 1-A shows the average urine output of 12 rats at room pressure (0 feet, the relatively unimportant height of the laboratory above actual sea-level being disregarded), followed by their average urinary output at 15,000 feet for the next 3 days. Figure 1-B illustrates the effect of exposing a fresh group of 12 animals to 3 daily periods at 10,000 feet *before* their first exposure to 15,000 feet. The results indicate that preliminary exposure to the lower altitude so acclimated the animals that 15,000 feet exposure no longer induced a polyuria; it was now necessary to reduce the pressure to 20,000 feet altitude equivalent before the first evidence of diuresis was observed. Raising the altitude to 25,000 feet and then to 30,000 feet in this series increased still further the observed polyuria (fig. 1-B).

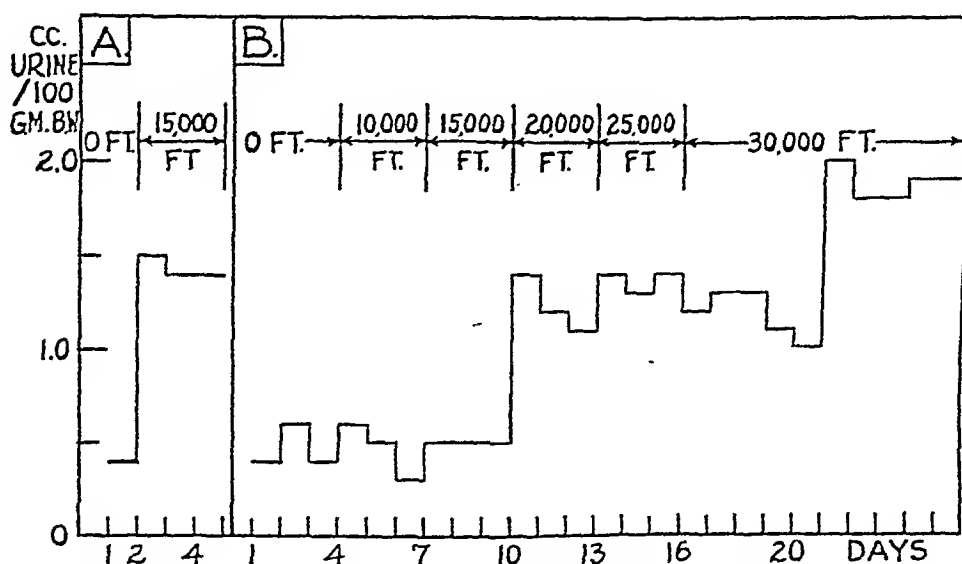


Fig. 1. Effect of 3-hour exposure to various high altitudes on urine excretion of white rats.

2. *Influence of temperature on urine and phenolsulphonphthalein excretion at high altitudes.*² Groups of 12 rats were injected intraperitoneally with 5 cc. per 100 grams of a solution containing 0.2 per cent sodium chloride and 2 mgm. per cent phenolsulphonphthalein and then placed in individual metabolism cages in the low-pressure chamber at an altitude equivalent of 15,000 feet. The chamber was maintained at a constant temperature of 10°, 20°, 30° or 40°C., and at the end of a 3-hour metabolic period the urine was measured and the dye determined colorimetrically. Results are given in table 1 as averages \pm probable error of the average. It will be seen that, regardless of the temperature from 10° to 30°, the animals reacted to 15,000 feet equivalent altitude by polyuria, which was further increased by cold but decreased by warmth. The dye excretion, however, was insignificantly affected, though it is questionable whether a phenolsulphonphthalein excretion time as long as 3 hours has more than comparative value. When the temperature was raised to 40°, practically all of the animals died within the experimental period, and those still surviving at

² These results were abstracted in part in Silvette (1943a).

the end were anuric. This was probably due to the high temperature and not to the low pressure involved.

To compare the effects of 15,000 and 25,000 feet altitudes, a series of 44 animals was injected as above and maintained for 3 hours at 25,000 feet. The average urine output at 25,000 feet was 3.7 ± 0.17 cc. per 100 grams as compared with 4.0 ± 0.12 cc. at 15,000 feet (both at $20^{\circ}\text{C}.$). Whereas in uninjected animals the 25,000 feet polyuria was much greater than the 15,000 feet diuresis (see section 1 above), in animals carrying a water load of 5 cc. per 100 grams body weight the 25,000 feet polyuria was less than that observed at 15,000 feet.

3. *Effect of anemic anoxia on urine output.* Before investigating the further effects of anoxic anoxia (due to decreased barometric pressure) on urinary output, the comparable effects of anemic anoxia (due to the inhalation of carbon

TABLE 1

Effect of various temperatures on urine and phenolsulphonphthalein output of white rats at high altitudes

NUMBER OF RATS	TEMPERATURE OF CHAMBER	0 FEET		15,000 FEET	
		Urine output	P.S.P. excretion	Urine output	P.S.P. excretion
	$^{\circ}\text{C}.$	cc./100 gm./3hrs.	per cent	cc./100 gm./3 hrs.	per cent
36	10	3.7 ± 0.13	66 ± 1.1	4.7 ± 0.12	65 ± 0.9
36	20	2.7 ± 0.09	64 ± 1.4	4.0 ± 0.12	70 ± 1.1
36	30	2.1 ± 0.08	55 ± 1.0	3.7 ± 0.14	61 ± 1.0
12	40	0.5		(dead)	

TABLE 2

Effect of carbon monoxide on urine and phenolsulphonphthalein excretion of rats

NUMBER OF RATS	EXPERIMENTAL CONDITIONS	URINE OUTPUT (AVERAGE \pm P.E. _{av.})	P.S.P. EXCRETION (AVERAGE \pm P.E. _{av.})
		cc./100 gm. B.W./3 hrs.	per cent
36	Normal control*	2.7 ± 0.09	64 ± 1.4
46	Exposed to carbon monoxide (0.4%) for 60 minutes	2.2 ± 0.08	56 ± 0.07

* See text.

monoxide) were determined. The 500-liter chamber containing 12 rats in individual metabolism cages (previously injected with fluid and phenolsulphonphthalein as in section 2 above) was sealed and partially evacuated, and the calculated amount of pure carbon monoxide gas was allowed to flow in to bring the CO-concentration of the enclosed air (now at room pressure) to 0.4 per cent. This concentration of gas was allowed to remain for 60 minutes before being replaced by fresh air. Four-tenths per cent carbon monoxide acting over one hour was sufficient to color the animals' skin (ears) the typical cherry-red in most instances, but was not lethal in any case or even seriously toxic, as judged by their general condition.

After a 3-hour metabolic period the urine was measured and the dye concentration determined colorimetrically. The results are given in table 2, compared

with control animals under similar conditions save for the absence of carbon monoxide. It will be observed that anemic anoxia brought about by carbon monoxide resulted in decreased urine and phenolsulphonphthalein output in contrast to the polyuria with normal or even increased dye excretion induced by anoxic anoxia due to exposure to low barometric pressure.

When the carbon monoxide concentration was further increased to 0.8 per cent there were many deaths, but in the still-living animals the oliguria was even more pronounced than in the case of rats exposed to 0.4 per cent concentration of the gas.

4. *Influence of preliminary water-load on urinary excretion at high altitudes.* Further experiments were designed to test the effect of various water-loads on urine output. As shown in table 3, the greater the water-load between 0 and 10 cc. per 100 grams body weight, the greater the *absolute* excretion of urine but the less the relative increase in urinary output of the animals maintained at 15,000 feet over their control (0 ft.) levels.

5. *Effect of repeated daily exposure to high altitudes on urinary excretion and kidney weight.*³ Fresh male rats maintained on a stock diet of Purina dog chow

TABLE 3

Effect of varying water-load on excretion of urine at 15,000 feet altitude equivalent

NUMBER OF RATS	WATER LOAD	AVERAGE URINE OUTPUT AT 0 FEET	AVERAGE URINE OUTPUT AT 15,000 FEET	INCREASE IN URINE OUTPUT AT 15,000 FEET OVER 0 FEET LEVEL
	<i>cc. 0.2 % NaCl/100 gm. B.W. i.p.</i>	<i>cc./100 gm. B.W./3 hrs.</i>	<i>cc./100 gm. B.W./3 hrs.</i>	<i>per cent</i>
36	0	0.4	1.0	150
36	5	2.7	4.0	48
36	10	3.9	5.0	28

and liberal amounts of greens, with water allowed *ad libitum*, were used. In groups of 12 they were weighed and placed in metabolism cages in the low-pressure chamber which was then evacuated to 15,000 feet altitude equivalent for a period of 3 hours. During this time the temperature was not controlled and generally varied from 23° to 28°. At the end of the 3-hour metabolic period air was admitted, the animals removed and their urine volume read and recorded. Three groups of animals were exposed to this simulated altitude for 3 hours daily for 17, 27 and 27 days respectively, and the average urine output of the first two groups is shown graphically in figure 2. The third group was sacrificed at the end of 27 days for histological examination and for the determination of organ weights. At the same time a control group of rats, maintained for the same period of time on the same diet and under similar environmental conditions, except for non-exposure to high altitudes, was sacrificed for control observations.

Out of the total of 36 animals given 3-hour daily exposures to an altitude

³ A preliminary report of the results at 15,000 feet equivalent altitude was given in Silvette (1942).

equivalent to 15,000 feet, only one died (on the 20th day). The remainder of the animals gained weight normally and appeared to be in perfect health throughout the course of the experiment.

The polyuria previously observed on single exposure of animals to 15,000 feet altitude was maintained throughout the long-term experiments at a generally high level. An initial marked diuresis observed in one group was not sustained (fig. 2-B), which may perhaps be taken as evidence of acclimatization; but at no time in the experiment did the urine output for the 15,000 feet metabolic period fall to that observed at room pressure. The other two groups did not show even this slight evidence of acclimatization, though in all three series the polyuric

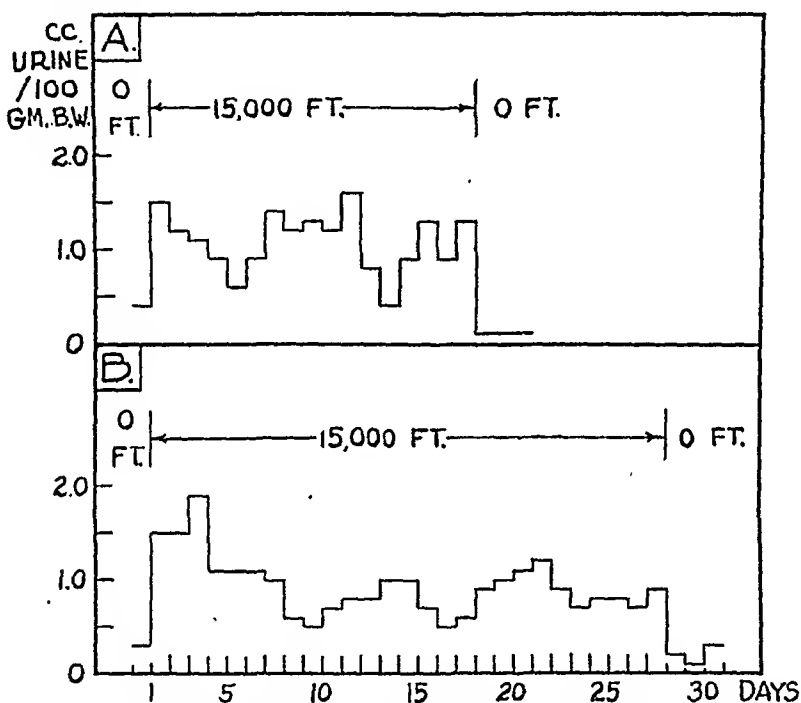


Fig. 2. Effect of daily 3-hour exposure to 15,000 feet altitude equivalent on urinary output of white rats.

response was cyclic, which may have been due to outside meteorological conditions prevailing throughout the course of the experiment.

When the animals were tested at room pressure following the determination of the period of 15,000 feet exposure, the urine output was observed to approximate that of normal animals never exposed to low pressures.

Two groups of 12 rats were similarly exposed for 3 hours daily to an altitude equivalent of 25,000 feet for 27 days each; the average urine output is shown graphically in figure 3. At the end of this time one of the groups was sacrificed for histological study and organ weights, together with a control group which had not been exposed to high altitudes. The remaining group was then followed for 8 days at room pressure and then re-exposed to 25,000 feet altitude for 3 hours daily for an additional 9 days (fig. 3-B).

There were no deaths in the first group of 12 animals exposed intermittently

for 27 days to 25,000 feet, and in the other group one animal died on the 8th day and one was sacrificed on the 27th day of the experiment. In contrast to animals exposed to 15,000 feet, which gained weight normally throughout the experiment, the 25,000 feet rats actually lost weight during the first few days of the experiment and only then began a slow moderate increase in body weight. This is demonstrated in table 4, which gives the average daily weight and average respiratory rate of the series described graphically in figure 3-A. The respiratory rate showed some evidence of adaptation and then acclimatization to the daily exposure to high altitude.

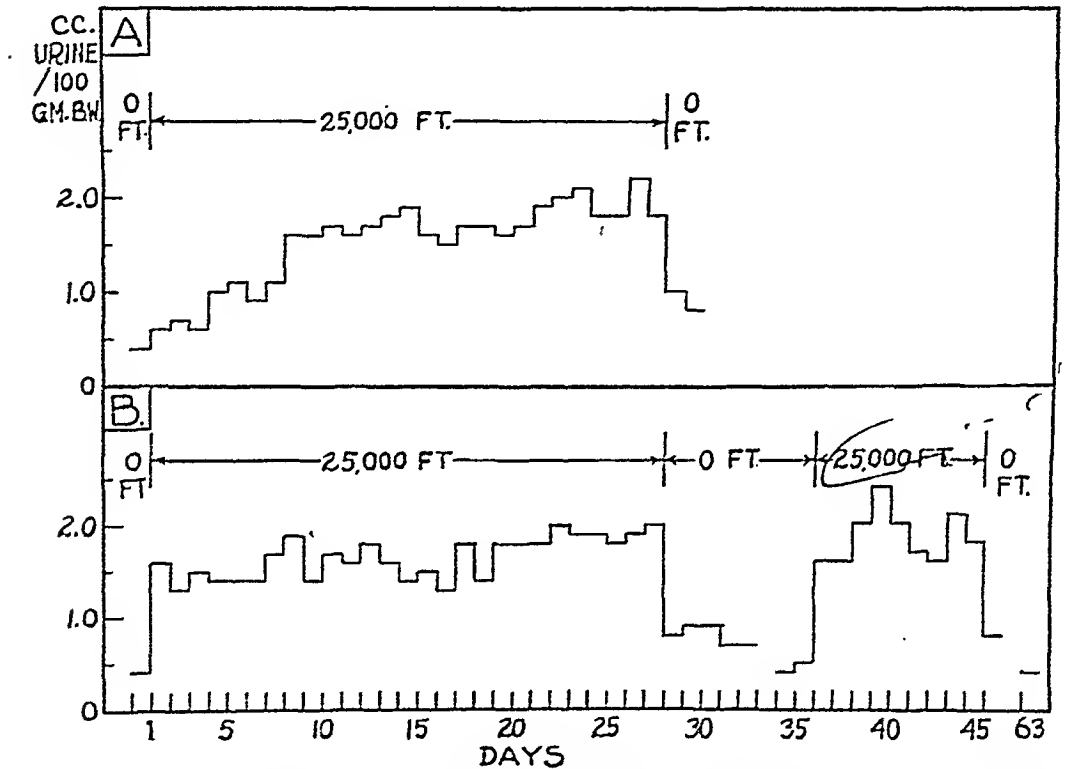


Fig. 3. Effect of daily 3-hour exposure to 25,000 feet altitude equivalent on urine output of white rats.

The polyuria observed in rats exposed daily to 25,000 feet altitude was even more pronounced than that recorded at 15,000 feet (fig. 3 and table 4). There was no evidence of acclimatization insofar as urinary output was concerned. In one group, indeed, the polyuria seemed to increase with time (fig. 3-A). Another significant difference between the two altitude effects was observable when the 25,000 feet group was tested at room pressure following the intermittent exposure to this altitude equivalent for 27 days. Immediately after long discontinuous exposure to 25,000 feet the average urinary output at room pressure was more than twice the urine excretion before the start of the experiment. Only after several days of recovery did the urine output return once more to the control level (fig. 3-B). This indicated that dysfunction in the case of the 25,000 feet group was more severe than that noted at 15,000 feet, a conclusion to which the two levels of polyuria lend contributory evidence.

Following the termination of the experiment as graphically outlined in figure 3-B, the animals were allowed to recover at room pressure under standard dietary conditions, and one month later were sacrificed for histological examination.

TABLE 4

Respiratory rate and urine output of 12 white rats exposed to 25,000 feet equivalent altitude for 3 hours daily

RUN	ALTITUDE EQUIVALENT	BODY WEIGHT (AVERAGE)	URINE OUTPUT (AVERAGE)	RESPIRATORY RATE* (AVERAGE)
	<i>feet</i>	<i>grams</i>	<i>cc./100 gm. B.W./3 hrs.</i>	<i>resp./min.</i>
1	0	192	0.4	78
2	25,000	190	0.6	
3	25,000	187	0.7	
4	25,000	186	0.6	
5	25,000	185	1.0	79
6	25,000	186	1.1	89
7	25,000	185	0.9	90
8	25,000	187	1.1	109
9	25,000	184	1.6	102
10	25,000	188	1.6	104
11	25,000	189	1.7	103
12	25,000	187	1.6	105
13	25,000	192	1.7	106
14	25,000	192	1.8	101
15	25,000	192	1.9	100
16	25,000	193	1.6	98
17	25,000	193	1.5	96
18	25,000	196	1.7	96
19	25,000	199	1.7	102
20	25,000	198	1.6	98
21	25,000	200	1.7	94
22	25,000	202	1.9	93
23	25,000	203	2.0	97
24	25,000	204	2.1	98
25	25,000	201	1.8	101
26	25,000	203	1.8	98
27	25,000	203	2.2	97
28	25,000	201	1.8	98
29	0	201	1.0	83
30	0	211	0.8	82

* Respiratory rates taken $\frac{1}{2}$ hr. after start of each experiment, at midpoint of exp., and $\frac{1}{2}$ hr. before end, and the 3 readings averaged to obtain resp. rate of each rat; thus each figure above represents average of 36 readings.

The relative weights of the kidneys in (A) normal control animals, (B) in animals exposed to 25,000 feet for 27 days to 15,000 feet altitude, (C) those of

of relative hypertrophy and the 25,000 feet exposure to a still more marked degree, but that recovery was followed by a fall of relative kidney weight below that of the initial normal controls.

Histologically, the kidneys of rats exposed for 3 hours daily to 15,000 feet altitude for 27 days appeared quite normal except for generalized hyperemia, but signs of beginning renal damage were observed after similar exposure to 25,000 feet. In the latter animals there was also renal hyperemia, and in addition, in several instances, necrosis of some peripheral tubules in the cortex. Pathological changes in the tubules, but accompanied by ischemia in place of hyperemia, were even more evident in the kidneys of animals allowed to recover at room pressure for one month following a similar period of 25,000 feet exposure (Kindred, 1943). The red cell count of the 15,000 feet exposure animals was essentially normal, but in those exposed to 25,000 feet for 27 days the erythrocyte count rose from a normal control level of 8,700,000 per cu. mm. to 11,700,000 per cu. mm.

TABLE 5
Effect of exposure to high altitudes on kidney weight

	NORMAL CONTROLS (0 FT.)		EXPOSURE TO 15,000 FEET FOR 3 HRS. DAILY, 27 DAYS		EXPOSURE TO 25,000 FEET FOR 3 HRS. DAILY, 27 DAYS		RECOVERY PERIOD, 1 MONTH AFTER EX- POSURE TO 25,000 FEET	
	Body weight	Weight of kidneys/ 100 gm. B.W.	Body weight	Weight of kidneys/ 100 gm. B.W.	Body weight	Weight of kidneys/ 100 gm. B.W.	Body weight	Weight of kidneys/ 100 gm. B.W.
	gm.	mgm.	gm.	mgm.	gm.	mgm.	gm.	mgm.
Maximum.....	300	965	275	1050	250	1,270	320	825
Minimum.....	210	770	225	850	160	890	249	680
Average.....	253	875	239	945	205	1,070	294	770
No. rats.....	15	15	11	11	12	12	8	8

6. *Effect of post-pituitary extract on the polyuria of high altitudes.*⁴ Experiments were performed to determine whether the polyuria observed at 15,000 feet could be reversed by the administration of post-pituitary extract. In one series of fully-fed animals injected with 5 cc. 0.2 per cent sodium chloride solution per 100 grams body weight, the 3-hour urine excretion at room pressure averaged 2.1 cc. per 100 grams. Animals similarly injected but exposed for 3 hours to 15,000 feet excreted on the average 5.0 cc. urine per 100 grams. Other animals, similarly treated but with the addition of post-pituitary extract (1.5 U.S.P. units per 100 grams B.W.) before exposure to 15,000 feet, averaged only 1.9 cc. urine in the 3-hour period, thus indicating that the polyuria of high altitudes was inhibited by the antidiuretic hormone.

Further animals were treated as follows with weekly rest periods between experiments: After a preliminary fasting period of 12 hours during which the animals were allowed water *ad libitum*, they were injected with 10 cc. per 100 grams 0.2 per cent sodium chloride solution with or without pituitary extract, and 3

⁴ These results have been given in abstract by Silvette (1943b).

hours later exposed in metabolism cages either to room pressure or to 15,000 feet altitude equivalent for a period of 3 hours. Results are given in table 6. In these experiments too the injection of post-pituitary extract reversed the high-altitude polyuria. Furthermore, the water retention brought about by equal doses of post-pituitary extract either at 0 or 15,000 feet was almost the same, i.e., 3.2 cc. at room pressure and 3.3 cc. at 15,000 feet. This would seem to indicate that the renal tubules were as sensitive to the antidiuretic hormone at low barometric as at room pressure.

DISCUSSION. Exposure to sufficiently high altitudes for 3-hour periods invariably led to polyuria in the white rat. At 10,000 feet altitude urinary excretion remained unaffected, as it did at 15,000 feet if the animals were first acclimated by preliminary exposure to 10,000 feet. But fresh animals exposed to 15,000, 20,000, 25,000, or 30,000 feet showed a marked diuresis which increased as the barometric pressure was lowered. In view of the reported results of other workers (Van Liere *et al.*, 1935; Toth, 1937) that dogs subjected to low oxygen tensions developed oliguria, it was at first thought that if the barometric pressure were sufficiently lowered, rats also would respond by reduced urinary excretion.

TABLE 6
*Effect of post-pituitary extract on high-altitude polyuria**

NUMBER OF RATS	POST-PITUITARY EXTRACT INJECTED	AVERAGE 3-HOUR URINE OUTPUT AT 0 FEET	AVERAGE 3-HOUR URINE OUTPUT AT 15,000 FEET
	<i>units/100 gm. B.W.</i>	<i>cc./100 gm. B.W.</i>	<i>cc./100 gm. B.W.</i>
36	0	3.9	5.0
36	1	0.7	1.7

* For details, see text.

However, rats exposed even to 30,000 feet altitude equivalent for 3 hours and dying of the exposure continued to exhibit the characteristic polyuria almost to the point of death. In other words, only moribund animals showed oliguria at high altitudes.

The polyuric effect of anoxic anoxia is entirely different from the effect on urine output observed in other types of anoxia. In anemic anoxia, due to carbon monoxide poisoning, the urinary output was reduced 18.5 per cent and the phenolsulphonphthalein excretion was reduced 12.5 per cent below the control levels, whereas anoxia due to low barometric pressure caused respective increases over the normal of 48 and 9.4 per cent. The stagnant type of anoxia, resulting from hemorrhage or circulatory failure, also leads to oliguria, as clinical experience demonstrates; and so does histotoxic anoxia brought about by cyanide poisoning (Beck, Kempton and Richards, 1938). Thus, of the four types of anoxia in Barcroft's classification, only anoxic anoxia leads to polyuria.

The water loss by way of the kidneys was so severe (1.0 to 1.6 cc. per 100 grams body weight in 3 hrs., exposure to 15,000–25,000 ft.) that it could not be expected that prolonged exposure to high altitudes would bring about an even greater diuresis. Indeed, the contrary is paradoxically true, for Collings *et al.*

(1943) report that young rats exposed for 12 to 24 hours to 380 mm. Hg (18,000 ft.) had urine volumes which were essentially identical with the controls. It is likely that the initial polyuria of the first few hours, which has been the invariable reaction shown by our animals, was compensated for by a subsequent oliguria, so that the urine volume over the 12-24 hour period was apparently normal. The results of certain of our own experiments lend credence to this belief: 36 rats were primed with 10 per cent of their body weight of fluid and 2.5 hours later were placed in metabolism cages for 9 hours. Urine output for the first 3 hours (experimental period) averaged 3.9 cc. per 100 grams body weight; for the second 3 hours (first recovery period), 1.1 cc.; and for the third 3 hours (second recovery period), 0.9 cc. Later, the same rats were similarly treated, except that they were exposed to an equivalent altitude of 15,000 feet during the 3-hour experimental period. The average urine output per 100 grams body weight during this experimental period was 5.0 cc. while at 15,000 feet, then 0.5 cc. during the first and 0.6 cc. during the second recovery periods. Thus it will be seen that, while the animals really showed the characteristic high-altitude polyuria when the two experiments were compared in detail, on the basis of the total 9-hour urinary excretion the controls excreted 5.9 cc. urine and the high-altitude rats only 6.1 cc., essentially identical figures.

The significance of the experiments on repeated exposure to high altitudes lies in three facts: that the polyuria was maintained for the duration of the experiments essentially unchanged; that following the termination of the period of exposure the urinary excretion returned to normal levels immediately or at the longest after a few days; but at the same time there was histological evidence of limited renal damage.

The increase in kidney weight relative to body weight after long discontinuous exposure to high altitudes was apparently not due to hypertrophy of any one part of the kidney at the expense of the other parts. The renal cortex and medulla retained their normal proportions to one another, and the tubules and glomeruli their normal diameters (Kindred, 1943).

While these differences in kidney weight reported herein are statistically highly significant, it must be conceded that the high-altitude hypertrophy and succeeding atrophy of recovery can be mathematically explained on the basis of loss or gain in body weight. However, from other considerations (such as the relation of kidney to body weight in normally-growing animals (Donaldson, 1924) and detailed histological studies in progress by Kindred (1943)), it is believed that the kidney weight changes observed bear a truly valid relationship to the functional changes (in urinary output) reported, though this relationship cannot be elucidated at the present time.

Other investigators have reported that long exposure to low barometric pressures led to a decrease in relative kidney weight. Sundstroem and Michaels (1942) noted a consistent decrease in the weights of kidneys of rats exposed continuously to pressures of 460 mm. (13,000 ft.), 360 mm. (19,000 ft.), 300 mm. (23,500 ft.) and 260 mm. Hg (27,000 ft.). Tornetta *et al.* (1943) reported that male rats exposed to pressures of 250 to 280 mm. Hg (25,000-28,000 ft.) for 6

hours daily for 14 to 18 days showed significant decreases in kidney weights. Eighteen to 20 hours' exposure for 3 to 12 days led to even greater kidney weight loss.

These experiments differed from those reported herein in that the daily exposure to high altitudes was much longer, or even continuous, whereas the number of days was usually less. Since the technique of measuring relative weights of such organs as the kidneys cannot be questioned, the discordant results obtained by the various workers must be a function of the different experimental conditions set up.

The reason for the high-altitude polyuria, like the explanation of the gain or loss in relative kidney weight, is still not clear. The relative glomerular hyperemia may be active, i.e., due to afferent arteriolar dilatation; passive, i.e., due to efferent arteriolar constriction; or secondary to polycythemia, though the latter was found only at 25,000 and not at 15,000 feet. This hyperemia may well have increased glomerular filtration somewhat, but the increase in urine excretion of 300 per cent over the normal at 25,000 feet was too great to be explained except by decreased tubular reabsorption as well. That there actually was tubular damage sufficient to bring about such decreased tubular reabsorption of fluid may be inferred from the histological evidence presented by Kindred (1943) and summarized in section 5 above.

SUMMARY

Exposure to sufficiently low barometric pressures for 3-hour periods invariably led to polyuria in the white rat. After exposure to 15,000 feet altitude equivalent the urine output was increased 150 per cent and after 25,000 feet exposure 300 per cent over the control (0 ft.) levels.

The high altitude polyuria was further increased by cold (10°C.), but decreased by heat (30°C.).

Phenolsulphonphthalein excretion was not significantly affected during exposure to 15,000 feet altitude equivalent.

In contrast to the polyuria with normal phenolsulphonphthalein excretion observed at high altitudes, anemic anoxia due to carbon monoxide poisoning resulted in decreased urine and dye excretion.

The greater the water-load, the greater was the absolute excretion of urine at 15,000 feet, but the less the relative increase in urinary output over the control (0 ft.) levels.

Repeated 3-hour daily exposures of white rats to simulated altitudes of 15,000 or 25,000 feet had no significant effect on their polyuric response, which was well sustained throughout the 27-day experiments. At the end of this time, when tested at 0 feet once more, the urinary excretion was normal or returned to normal within a few days. Relative kidney weights of these animals indicated a slight degree of renal hypertrophy after the period of 15,000 feet exposure, a more marked degree of hypertrophy after 25,000 feet exposure, but an apparent atrophy after a one-month recovery period at room pressure. The histological

picture of the kidneys of animals after long intermittent exposures to 15,000 feet was normal, except for marked hyperemia, but at 25,000 feet beginning slight pathological changes occurred.

The polyuria of high altitudes may be inhibited or reversed by the injection of post-pituitary extract.

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THE EFFECTS OF LOW BAROMETRIC PRESSURES ON THE STRUCTURE OF THE KIDNEYS OF THE WHITE RAT

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Silvette (1) in a recent paper reported a greater output of urine in rats subjected for three hours daily for 27 days to low barometric pressure (428.8 mm. Hg, equivalent to altitude of 15,000 ft.) than in the same rats and in control groups maintained at room pressure. Since this publication, Silvette has carried his experiments further and has subjected rats to lower barometric pressure (equivalent to 25,000 ft. altitude) for the same periods of time. He reports a further increase in urine output as compared with the rats at room pressure (fig. 1) and at pressure equivalent to 15,000 ft. altitude (2). Silvette observed that accompanying the increased urine output there was a relative increase in the weight of the kidneys per 100 grams of body weight. Since no histologic studies have appeared concerning the kidneys of animals so treated, the animals were turned over to me following these experiments with the request that the kidneys be examined for morphologic changes which could be related to the functional changes and the changes in weight of the kidneys which were reported. The important physiologic change to which the experimental rats have been subjected is anoxia.

MATERIALS. Three groups of living rats, all males, of Wistar strain, but not litter mates, were turned over to me for study immediately after the experiments. Group I, controls, 15 rats, average weight 250 grams (range, 210–300 grams); group II, 11 rats which had been exposed to barometric pressure equivalent to 15,000 ft. altitude for 3 hours daily for 27 days, average weight 236 grams (range, 230–275 grams); group III, 12 rats which had been exposed to barometric pressure equivalent to 25,000 ft. for 3 hours daily for 27 days, average weight 205 grams (range, 160–250 grams). These rats were killed with ether. Blood counts were made from the femoral artery just before death. Following death the kidneys and certain other organs were removed and weighed to the nearest centigram.

The kidneys were either split in the sagittal axis or transverse to the long axis and fixed in either Helly's fluid or in 10 per cent formalin. The usual paraffin technic of embedding and sectioning was followed. Sections were cut at 7 microns and stained either with hematoxylin and eosin or with Kornhauser's quadruple stain (3). The latter is an excellent stain, because the red blood corpuscles, tubules and connective tissues are sharply differentiated from each other.

In addition to this material, eight male rats, average weight 294 grams (range, 249–320 grams), which had been exposed 3 hours daily for 27 days to 282 mm. Hg pressure (equivalent to 25,000 ft. altitude) following which they had been

kept at room pressure for one month, were killed by Doctor Silvette and the kidneys were removed, weighed and fixed in 10 per cent formalin and given to me. The histologic technic described above was applied to these kidneys. These will be designated as rats of group IV, and the others as groups I, II and III, respectively, in the following description.

Observations. The average weights of the kidneys per 100 grams of body weight with their standard errors were as follows: Group I (controls); 875 ± 15 mgm. Group II (15,000 ft.); 945 ± 19 mgm. Group III (25,000 ft.); 1070 ± 36 mgm. Group IV (one month after 25,000 ft.); 770 ± 17 mgm.

The data on the weights of the kidneys of the rats of groups I and II have been used by Silvette (1) in support of his view that increased urine output is accompanied by relative increase in weight of the kidney; and those of groups III and IV in a paper in press (2). Statistical contrasts showed that all weights

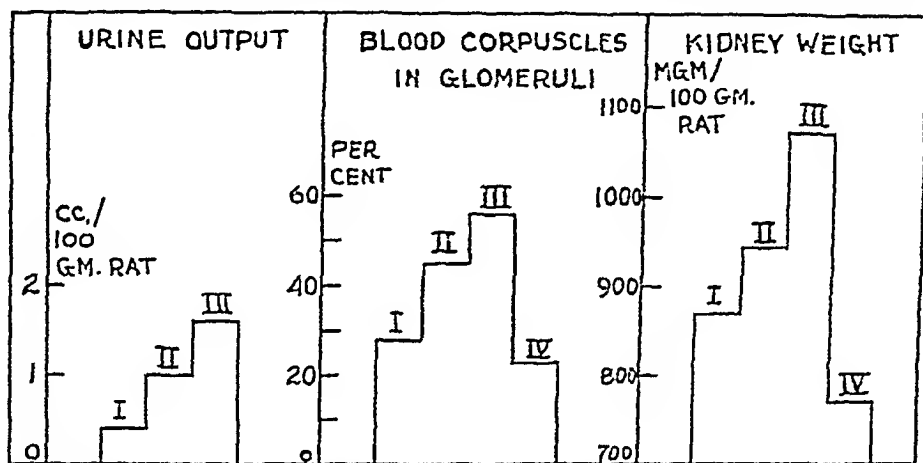


Fig. 1. Graph showing correlation between average urine output per 100 grams of body weight; average kidney weight per 100 grams body weight; and average per cent of red blood corpuscles per glomerulus of kidneys of rats maintained at barometric pressures corresponding to different altitudes. I, 0 ft., (controls); II, 15,000 ft.; III, 25,000 ft.; IV, 0 ft., one month after exposure to 25,000 ft. Data on urine output from Silvette (2).

are significantly different from each other and that weight increases as the barometric pressure decreases. The kidneys of the rats of group IV showed a very great decrease in relative weight when contrasted with the kidneys of groups II and III, and a significant but relatively lower weight than the kidneys of the rats of group I.

Examination of the sections of the kidneys revealed certain qualitative and quantitative morphologic changes which are thought to be relevant to the changes in the weights of the kidneys and to the functional polyuria observed in the experimental groups. Low magnification studies were made of sections of the kidneys of groups I and II in which the proportions of areas of cortex and medulla in a segment from approximately the same region of the kidneys were compared. No differences were observed in the relative amounts of cortex and medulla in the two groups. Counts of the glomeruli per unit volume and measurements of the sizes of the glomeruli were also made on the kidneys of these

two groups of rats using higher magnification and no differences were found. The average diameters of the tubules were found to be the same in both groups. Since the method of making these measurements is rather time consuming and since the results were negative, the measurements were not done on the rats of groups III and IV.

During these examinations of the kidney with the higher magnification (1200 diameters) it was observed that while it was quite difficult to see the intertubular capillaries in the cortex of the control kidneys (Bensley, 4, has recorded the same conclusion from study of rabbit kidneys), these capillaries were quite easily visible by reason of their distention with red blood corpuscles in the kidneys of the rats of groups II and III. In these kidneys the "vascular rays" of Bensley (4), the terminations of the efferent arteriolae rectae of the glomeruli, stood out more sharply and seemed to contain more blood than similar channels in the kidneys of the controls. Since these arteriolae rectae are directly connected with the glomerular capillary network these regions were next examined. Casual observation suggested that there was hyperemia and distention of the capillaries in the glomeruli of the kidneys of groups II and III when these were contrasted with those of groups I and IV. Such observations led to the application of a quantitative method for measurement of these conditions. The paper weight method which Jackson (5) used in estimating the comparative proportions of the stroma and parenchyma of the adrenal glands was applied. The outline of a median vertical section of a glomerulus was drawn with camera lucida on paper. Within the glomerulus the areas occupied by red blood corpuscles within the glomerular capillaries were outlined. Then the outlined glomerulus was cut out and weighed to the nearest centigram, following which the areas outlining the blood corpuscles were cut out and weighed. The percentage of red blood corpuscle volume per glomerulus was estimated from these weights. In order to see if this were a representative sample of the volume of the red blood corpuscles in the whole glomerulus, the average percentage of red blood corpuscle weight measured in serial sections of 10 glomeruli was compared with the average value obtained in only the midvertical sections of these glomeruli. The average weight of the red blood corpuscles for the whole glomeruli was 23 ± 1.9 per cent of the glomerular weight, and for the midvertical sections, 23 ± 1.7 per cent. From this contrast it was felt that the measurements of the midvertical sections gave an accurate measurement of the total weight of the red blood corpuscles in the glomeruli.

The results of these measurements are summarized in table 1, in which the percentages of red blood corpuscle weight in the glomeruli are tabulated by percentage classes in the different groups of rats. Examination of table 1 shows that in group I (controls) the modal classes are those in which the red blood corpuscles occupy between 21 and 40 per cent of the glomerulus; in group II (15,000 ft.) the modal classes extend from 31 to 50 per cent; in group III (25,000 ft.) from 51 to 70 per cent; and in group IV (one month after 25,000 ft.) from 11 to 30 per cent. This table shows that by the method used there is evidence of an increase in the amount of red blood corpuscles in the glomeruli

with decrease in barometric pressure and that when the rats are removed from this environment and kept at room pressure for one month there is a sharp drop in the amount of blood present.

Further statistical analysis in which the means of the blood corpuscle percentages with their standard errors of the several groups were contrasted shows the following results: Group I, average blood corpuscle percentage 28 ± 0.65 per cent. Group II, average blood corpuscle percentage 45 ± 1.3 per cent. Group III, average blood corpuscle percentage 56 ± 0.81 per cent. Group IV, average blood corpuscle percentage 23 ± 0.93 per cent.

Difference between groups I and II, 17 ± 1.48 per cent; t 11.5. Difference between groups II and III, 9 ± 1.5 per cent; t 6.0. Difference between groups I and IV, 5 ± 1.12 per cent; t 4.45.

TABLE 1

Distribution by classes of percentage of red blood corpuscles in the glomeruli of kidneys of control rats and of rats exposed to low barometric pressures

I, controls; II, 15,000 ft.; III, 25,000 ft.; IV, one month after 25,000 ft. The percentages of the red blood corpuscles were measured in midvertical sections of ten glomeruli from each kidney; number of kidneys: I, 10 from 10 rats; II, 9 from 9 rats; III, 11 from 11 rats; IV, 8 from 8 rats. Total number of rats, 38; total number of kidneys, 38; total number of glomeruli, 380.

PER CENT	GROUP			
	I	II	III	IV
1-10	0	0	0	2
11-20	16	0	0	33
21-30	49	1	0	30
31-40	32	30	6	15
41-50	3	38	24	0
51-60	0	17	45	0
61-70	0	4	34	0
71-80	0	0	1	0

Since the t ratio in all these contrasts is larger than 2.5, the differences are considered to be greater than could occur by chance and hence are statistically significant (6). These data establish the fact that changes in the environmental barometric pressure produce measureable effects on the amount of red blood corpuscles in the glomerular capillaries.

Such changes in the amount of blood corpuscles could very well account for part of the increased diuresis which accompanies the exposure if the results are interpreted according to the suggestion by Drinker and Yoffey (7) which is as follows: "It is conceded that capillaries in most places in the body experience change in diameter and that these changes constitute an important regulatory feature of many physiologic reactions such for example as muscle work, formation of urine, etc." In this particular group of experiments the capillaries in the glomeruli of the kidneys of the experimental rats are distended with red blood corpuscles and such distention could accordingly lead to increased glomerular

capillary pressure and increased capillary permeability, an interpretation which is suggested by the conclusion of Landis (8) that permeability of the endothelium of the capillary is not determined by the capillary diameter, but by capillary pressure.

It has been suggested by Richards and Schmidt (9) that the blood flowing through the glomerular capillaries is controlled by an efferent constrictor mechanism and it has been shown by Bensley (4) in the rabbit and guinea-pig kidneys that there are cells (pericytes) arranged in circular fashion around the efferent arterioles which conceivably could perform this function. Hence the sections of the hyperemic kidneys were examined to see if the hyperemia of the glomeruli could have been produced by contraction of such cells. Serial sections of glomeruli were examined and the efferent arterioles were found to be widely dilated and directly continuous with the enlarged intertubular capillaries. Hence the dilatation of the capillaries in the glomeruli can only be viewed as one effect of a relaxation of the whole renal vascular system. For not only were the intertubular capillaries enlarged, but the archiform veins were also more distended than were those in the kidneys of the control rats. Hyperemia, then is characteristic of the whole kidney and since it is present in rats which have been exposed to anoxic conditions it may logically be concluded that the hyperemia is the result of the anoxia, but whether it has been brought about by local tissue response or through a central mechanism cannot be decided by the evidence presented here. Furthermore, it seems logical that the polyuria characteristic of these rats is partly the result of increased glomerular pressure and greater capillary permeability, although as Silvette (2) has suggested, the observed polyuria was too great to have been the result of increased glomerular pressure alone. The correlation between the output of urine and the percentage of red blood corpuscles in the glomeruli is shown in figure 1. No statistical analysis was made of this correlation, hence it can only be suggestive.

Another view which might be taken is that the rats have been so affected by the anoxic conditions that there has been an increase in the number of red blood corpuscles per cubic millimeter and that the hyperemia observed in the glomeruli is the result of anoxic polycythemia. An analysis of this possibility showed that neither in group I (controls, ave. rbc. per cu.mm., 8.65 millions) nor in the rats of group II (15,000 ft., ave. rbc. per cu.mm., 7.7 millions) is there any correlation between glomerular hyperemia and the rbc. count. In the rats of group III (25,000 ft., ave. rbc. per cu.mm., 11.7 millions) there is a suggestive positive correlation, but it is not considered statistically significant. Therefore, it is concluded that such polycythemia as may occur in the rats of group III is not sufficient to account for the renal hyperemia observed.

As has been stated in the introduction, Silvette (1) observed that increase in relative weight of the kidneys was accompanied by increased output of urine. The question might now be asked whether there is any correlation between hyperemia and the weight of the kidneys. Statistical analysis of this question, using the kidney weight per 100 grams of body weight and the percentages of red blood corpuscles in the glomeruli as the contrasting values, has shown that

for the whole population, pooling the weights of the kidneys of rats in all groups, the moment of correlation as derived using the methods of Fisher (6) is 0.655. Such a value implies that the correlation is significant of an association which is better than could occur by chance. This correlation is shown in figure 1. Since the glomerular hyperemia is one part of the total renal hyperemia which is characteristic of the kidneys of the experimental rats it is possible to conclude, using the established correlation as an indicator only, that the observed increase in kidney weight in rats exposed to low barometric pressures could be accounted for by the additional blood present.

In addition to the changes which can be called normal, it was observed that in the kidneys of the rats of group II (15,000 ft.) the histology of the kidney was unchanged except for the hyperemia. In the kidneys of the rats of group III (25,000 ft.) damage appears to begin. As in the rats of group II, the predominant picture is one of hyperemia of the glomeruli, intertubular capillaries and veins. However, in some of the kidneys there were small areas of necrosis of tubules in the cortex and one kidney showed a small area of tubule and glomerular shrinkage. In kidneys of six of these rats there were small areas of lymphocyte infiltration, but since similar areas were found in some of the kidneys of the control group, this condition is not considered to be significant. In one kidney of one rat there was an accumulation of neutrophilic granulocytes in the hilar fat subjacent to the renal sinus mucosa.

In the kidneys of the rats of group IV, the necrosis observed in some of the kidneys of rats of group III was present in kidneys of five of the eight rats studied. There was shrinkage and flattening of the glomeruli and peripheral tubules in some parts of the cortex in the kidneys of all of these rats. The kidneys were extremely ischemic and no blood was seen in the intertubular capillaries or in the vascular rays. As has been pointed out above the glomeruli were notably deficient in blood content. Calcareous deposits were present in the tubules of the cortex of one kidney. In four of the eight kidneys, neutrophilic granulocytes were massed in the hilar fat subjacent to the renal sinus mucosa. There was fibrosis in the region of the vascular rays in one kidney. On the whole, the kidneys of this group showed distinct pathologic changes when contrasted with the kidneys of the other groups.

From these observations on kidney damage it may be concluded that the kidneys of rats can stand the anoxia when subjected to it, but the degree of anoxia (concomitant with lowered barometric pressure) produces a latent condition of parenchymatous damage which appears to become extensive after the rats have been removed from the anoxic condition for some time. More study should be given to this effect as the possibility of permanent kidney damage resulting from chronic exposure to anoxic conditions whether in industry or in aviation has not been realized.

SUMMARY

The kidneys of rats which had shown polyuria while exposed to low barometric pressure daily for 3 hours for 27 days (1, 2), show relative increase in weight,

hyperemia and a slight degree of parenchymatous damage. There is a positive correlation between hyperemia and the increase in weight of the kidneys. It is suggested that diuresis is related in part to increased permeability of the glomerular capillaries because of the increased glomerular pressure assumed to accompany capillary distention. The direct effect of anoxia (which accompanies the low barometric pressures) on the kidney vasa is suggested as the cause underlying hyperemia.

The kidneys of rats which have been returned to room pressure for a month following the exposure to low barometric pressure show extensive parenchymatous damage, calcification, shrinkage of the stroma, and ischemia.

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THE VALIDITY OF "OVULATION POTENTIALS"¹

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An increase in the difference of potential between a vaginal and a suprapubic (surface) electrode at the time of ovulation was first noted by Burr, Hill and Allen (1) in the rabbit. This finding was confirmed by Reboul, Friedgood and Davis (2, 3). In the rat similar electric changes at the time of estrus were observed by Rogers (4-6). Employing surface electrodes on abdominal and flank areas respectively, Altmann (7, 8) reported marked voltage gradients in the hen at the time of ovulation, and in the sow simultaneously with external signs of estrus.

In the human female, Burr et al. (9, 10), using an abdominovaginal circuit, described the same phenomenon during ovulation time in one case as did Rock, Reboul and Wiggers (11) also in one patient. In 1938, Rock, Reboul and Snodgrass (12) repeated these experiments on nine other women during the theoretical ovulation period (based on catamenial history). In some of these experiments, however, a pair of surface electrodes, applied at various levels between the umbilicus and the symphysis pubis was used, alone, or in conjunction with a second surface pair, or with an abdominovaginal circuit.

When the pertinent data of all ten cases of Rock and his associates (11, 12) were analyzed, a number of inconsistencies were noted in the results obtained. In the first place, regardless of the type of set-up: whether abdominovaginal electrodes or surface electrodes were used, or whether both types of circuit were employed simultaneously, the potential change was noted in some cases even before ovulation had occurred. Therefore a statement was made in the original report (12) that the change was only *associated* in some way with ovulation.

In the second place, there were wide and inexplicable variations in the magnitude, duration and polarity of the electric changes.

Furthermore, unaccountably erratic results were obtained when surface electrodes were employed simultaneously with an abdominovaginal circuit. In one case, definite changes were recorded in the surface circuit in the absence of any change in the abdominovaginal system.

If these "ovulation potentials" are all due to electric activity in the ovary associated with the process of ovulation (3), it is difficult to see how the potential changes can increase in magnitude as the electrodes are moved further away from the ovary. In one patient tested, the change in potential between a pair

¹ Aided by grants from the American Academy of Arts and Sciences, the William F. Milton Fund of Harvard University, the Committee for Research in Problems of Sex, National Research Council, and Mr. Richard C. Paine.

of surface electrodes placed low in the pelvis was approximately three times as great in magnitude as that between the abdominovaginal pair. Moreover, in all but one case, the highest values obtained were those recorded from surface electrodes.

An increase in magnitude of the difference in potential as the electrodes were moved away from the ovary was also noted by Reboul, Davis and Friedgood (3). In addition, Rogers' (6) finding of a similar change in difference of potential on the last day of estrogen-induced estrus in the spayed rat further increased our skepticism as to the relation between the so-called "abdominovaginal sign" and ovulation. It seemed evident that some hitherto unknown factor must be concerned in the production of these widely variable results.

In order to ascertain the nature of this factor, it was deemed essential, first of all, to determine whether one could locate a point on the surface which would be stable in potential and could thus serve as the site for a reference electrode. With this purpose in mind, the abdominal region and thighs of six human subjects were mapped in terms of electric potential.

I. SKIN POTENTIALS IN RELATION TO THE "ABDOMINOVAGINAL SIGN" OF OVULATION. *Surface Potentials: Isopotential Contour Patterns. Method.* The reference electrode consisted of a large Ag-AgCl plate about 45 sq. cm. in area. This was firmly fastened just above the left knee by means of a loose elastic binder. A layer of cotton soaked in physiologic saline solution was placed between the chlorided silver sheet and the skin. The exploring electrode, also of Ag-AgCl, had an effective contact area of about 16 sq. mm.; here, too, a small piece of cotton moistened with physiologic salt solution made the actual contact with the skin.

The difference in potential between the electrodes was measured by means of a null method. The effective input resistance to the measuring circuit was 2 meg. ohms. The null point was usually quite sharp, and currents less than 5×10^{-10} amp. flowed in the electric circuit. The unbalanced potential was shunted by a vibrating contact at the grid of the first tube of a high-gain audio frequency amplifier. The output of the amplifier operated a pair of sensitive head-phones. Fairly accurate potential determinations could be made in 3 to 5 seconds.

Results. On each individual studied, 75 to 100 determinations of potential were made. When the potentials were sufficiently stable, it was possible to construct a clearly defined series of isopotential contours, as shown in figure 1. No demonstrable effect of sex on isopotential contours was noted.

In general, a focus of potential may be found on the thighs in the region where the femoral artery and vein are most superficial. Potential foci may be observed also at almost any point on the abdomen and do not seem to have any demonstrable connection with underlying organs.² As a rule, there are relatively few or no potential foci in the suprapubic area.

² On one individual a very interesting observation was made which tends to indicate further the cutaneous nature of these potentials. In plotting the isopotential contours of any subject, the potential value of a particular area was written on the skin by means of a stub-pointed fountain pen, since the latter required less pressure than the usual skin pencil and caused little or no disturbance in the potentials. One subject happened to exhibit marked dermatographia, and it was strikingly clear that the more positive the potential of the area, the higher were the wheals produced by the pen-point.

On certain individuals, potential foci and isopotential contours were very stable and the same pattern was obtained at intervals as great as two weeks. Other individuals had such unstable potentials that even a small abdominal area could not be plotted. In general, persons tending toward the "sthenic" type seemed to be unsuitable as subjects for isopotential contour plotting.

In three subjects, measurements were made during the estimated ovulation time while they were awaiting operation. If ovulation is accompanied by a fairly large electric potential change in the ovary, such a shift in voltage might reasonably be expected to appear in the isopotential contour pattern. Yet even

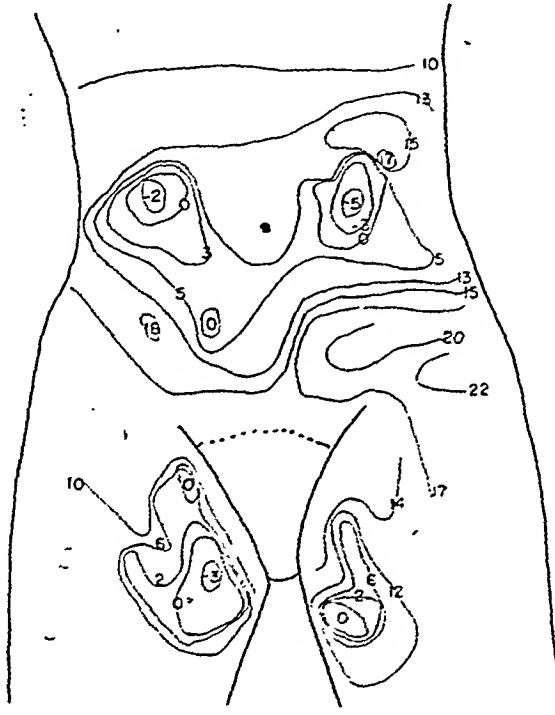


Fig. 1. Isopotential contours measured on a male subject. Potentials plotted in millivolts. For measuring purposes a large reference electrode was placed just above the knee. Potentials plotted with respect to arbitrary value of zero for potential focus in left femoral region. The voltage range shown is from +22 mv. to -5 mv. Note the steep potential gradient near the lower left boundary of the epigastric region. The potential ranges from -5 to +17 mv. within an actual distance of 4 cm.

in the presence of electric instability, no clear potential foci were found which could be related to the underlying ovaries. As far as could be determined, the reason for the instability of the electric potential was due entirely to the emotional uneasiness of the subjects.

Direct Measurements of the Ovary. Still further to check the ovary as a possible source of potential, direct electric measurements were made on human ovaries *in situ* of three women during the course of routine laparotomy. In each case the operation was performed within the patient's estimated ovulation time.

Method. The same leads and measuring equipment as described above were used, except that for the present experiments we devised a special unipolar exploring electrode of Ag-AgCl agar which could be autoclaved. This was made by plugging an 8-inch length of $\frac{3}{16}$ -inch glass tubing at one end with a loose roll of filter paper about $\frac{1}{2}$ inch long. The tube was filled with a hot agar-saline solution, and a 4-inch length of no. 20 B. & S. gauge chlorided silver wire was pushed down into the agar. The silver wire was soft-soldered to a fine multi-stranded copper wire which, in turn, was pulled through an 8-foot length of $\frac{1}{8}$ -inch pure gum tubing ($\frac{1}{16}$ -inch bore). The lead was fastened to the top of the electrode tube by means of rubber bands. The electrode and its lead wire were inserted in a 10-inch glass cylinder. The latter was plugged with gauze in the usual manner, and the assembly placed in the autoclave in an upright position. For convenience, the potential determinations were referred to the broad ligament as zero.

Results. In general, no potentials greater than ± 1 mv. were found between any two organs in the abdominal cavity. Several follicular surfaces (including that of one fairly large, presumably mature follicle³), as well as the intervening ovarian cortex, were tested, but no significant electric potential was detected. The highest potential recorded, slightly less than $+2$ mv., was obtained from a locus on the *Rectus abdominis* muscle.

II. FACTORS INFLUENCING "FINGER-TO-FINGER POTENTIALS."⁴ Several investigators have recorded an increase in the difference of potential between the index fingers of women at various times during the menstrual cycle, and have concluded that such a change indicated ovulation (14-19). Barton (17) deduced from her data that follicular rupture might take place at any time, even during the menses, and sometimes twice in the same cycle, while Altmann (19) reported that ovulation might occur between the 5th and 23rd days.

Since the establishment of a clearly defined criterion of ovulation time would be most important, both from a theoretical and from a clinical viewpoint, we undertook in 1939 similar experiments on 30 nurses (group 1) and 23 female employees (group 2) of the Free Hospital for Women. In addition to women whose cycle lengths were within normal limits, the subjects included also one surgical castrate, one woman in natural menopause, one pregnant individual, and two women who, on the basis of their clinical history, doubtless had anovulatory cycles (20).

With the exception of Sundays, measurements were made daily before lunch on all subjects during the months of April and May. In addition, one of the two groups of subjects was also studied during the last week of March.

It should be noted that, in order to rule out any factors which might unconsciously influence the experimenter, the latter knew nothing about the menstrual dates or clinical history of the subjects. At the beginning of the experimental period, the women were all given printed calendar cards on which to record their catamenial dates and any other pertinent information. The cards were collected by the investigator only at the conclusion of the experimental period.

³ At present we know of no reliable way to determine by gross examination alone whether a large follicle is normal or atretic.

⁴ A preliminary report of this work was presented before the American Physiological Society, New Orleans, 1940 (13).

Method. The potential determinations were made with the null-measuring system described above.⁵

Experience with meter-indicating types of vacuum tube voltmeters shows that it is frequently almost impossible to determine whether or not a particular meter reading is a true indication of the real potential. Occasionally the readings have been found to be in error by several hundred per cent. This may be due to several causes, the most important of which is the difficulty of shielding the subject completely from stray 60-cycle electric fields. Radio frequency fields may be particularly troublesome, as some element in the external circuit, or the vacuum tube itself, may rectify the high frequency and produce deflections of the meter. This is especially true in hospitals where diathermy machines are employed. The particular type of null measuring equipment used to obtain the data presented in this paper possessed the advantage of giving an immediate acoustic clue as to the probable source of spurious potentials, and hence proper precautions could be taken in order to obtain a correct determination of voltage. The measuring equipment was also free from the erratic and disturbing influences of electrostatic charges, however generated.

The fingers were immersed in beakers of saline solution, in the manner described by Burr and Musselman (14). However, unlike investigations of other workers, where measurements were confined to the index fingers, determinations in the present experiments were made between *each* pair of fingers, excluding the thumb; that is, pairs of index fingers, middle fingers, etc., were tested separately. The potential readings from the four pairs of determinations were averaged for each subject, and this individual mean was used in calculating the average for each of the two large groups (group 1, nurses; and group 2, employees).

Finger Potentials in Relation to the Presence or Absence of Normal Menstrual Cycles. The upper plot of figure 2 depicts the day to day changes in potential between each pair of fingers of a subject (case 66) with normal menstrual cycles. There is no evidence of any cyclic variation with reference to the onset of catamenia, nor do these data demonstrate any unique property possessed by the index fingers (no. 2). In general, our findings confirm the statement of Parmenter (16) that there is "no consistent unidirectional polarity of the right index finger."

The lower plot of figure 2 represents the finger potentials of a normal menopausal individual (case 87). There is no significant difference between this graph and the upper one. Furthermore, the presence of minor excursions in the plot from the menopausal subject serves to indicate that similar fluctuations recorded for no. 66 are not pertinent to menstruation.

Likewise, data from one pregnant subject, one surgical castrate, and two women who doubtless had anovulatory cycles, did not differ from records of those with normal menstrual cycles.

No significant differences in the finger potential curves of a cyclic nature were observed between individuals. Some subjects tended to show a greater day to day variation than others, but this remained a constant factor. As in the case of the isopotential contour determinations reported above, the individuals who exhibited the greater day to day variations of potential were those of the "sthenic" type.

⁵ In the experiments on finger potentials reported by others (14, 15, 17, 18), the Burr-Lane-Nims vacuum tube voltmeter was used (21). Parmenter (16) also employed a meter-indicating type of vacuum tube voltmeter.

From a consideration of these individual curves, we have been completely unable to confirm the observations of other workers who have reported a significant rise of potential between the index fingers associated with ovulation.

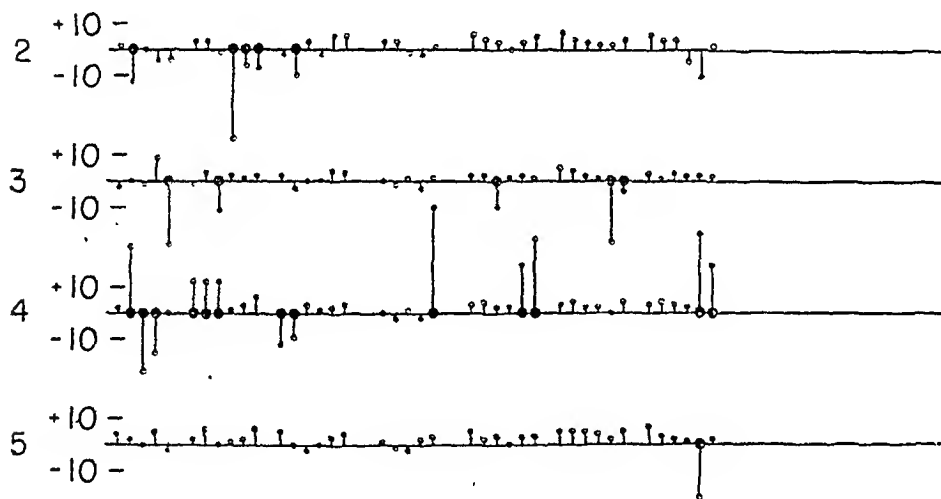
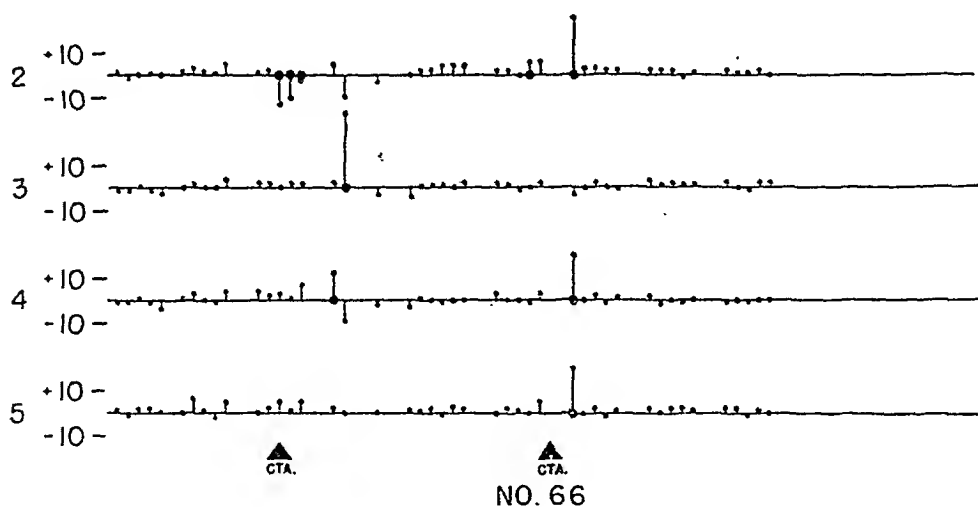


Fig. 2. *Upper plot.* Daily finger potentials, plotted in millivolts, of a normal, healthy female (no. 66). The left finger of the pair being measured was arbitrarily used as the reference finger. The potentials are plotted for each pair of fingers except the thumbs (# 1 fingers). It was not practical to use the thumbs due to the prevalence of cuts, bruises, etc. The solid black circles on the time axis indicate when the potential measured was considered invalid due to skin damage, such as cuts and hangnails. The solid black triangles represent the beginning of the menses. It is quite apparent that the index fingers (# 2) are not unique in so far as potential changes are concerned.

Lower plot. Daily finger potentials of a normal menopausal subject (no. 87). Data obtained concurrently, with the same equipment and in the same way as those shown in the upper graph. The solid black circles here also represent potentials as measured, but considered invalid due to skin damage. There is no significant difference between the two plots shown in this figure. The greater number of invalid potentials recorded in the lower graph is probably due to the fact that subject 87 performed considerably more manual work than subject 66. The hands and fingers of the former were injured very frequently.

Magnitude and Polarity of Potential Changes. When great care was taken to check spurious finger potential measurements, no voltage reading higher than 12 mv. was found to be valid. Moreover, only three or four out of approximately 14,000 determinations showed this high figure. Usually 7 mv. represented the maximum value.

All potentials higher than 12 mv., and many lower than this, were found to be spurious because of cuts, pin-pricks, hangnails, bruises, etc. It must be strongly emphasized that it is practically impossible to detect visually—even with the aid of a magnifying lens—all types of skin damage. We found ethyl alcohol (95 per cent) to be more dependable as a means of detecting abrasions, for even a slight one will cause a stinging sensation upon immersion of the fingers in this fluid.

The average finger potential of the combined groups of nurses and employees was +1.7 mv. Since the left hand was used as the reference side, this means that the fingers of the right hand averaged 1.7 mv. positive with respect to the

TABLE 1
Average monthly finger potentials of female subjects in relation to "handedness"

	SUBJECT NUMBER	APRIL AVERAGE	MAY AVERAGE		SUBJECT NUMBER	APRIL AVERAGE	MAY AVERAGE
		mc.	mc.			mc.	mc.
Left-handed	67*	-1.20	-0.68	Right-handed	51	+2.11	+1.40
	78†	-0.31	-0.29		54	+3.71	+5.00
	80	-4.16			60	+4.40	+4.13
	102	-0.81	-0.57		63	+3.11	+3.89
					67	-1.20	-0.68

* Although normally right-handed, this subject was doing filing work which necessitated pulling and pushing heavy file drawers with her left hand. She was therefore included in both groups.

† This subject, whose finger potential was very near zero, was a nurse who assisted in the operating-room and was notably ambidextrous.

fingers of the left hand, a finding in reasonable agreement with the figure of 2.25 mv. published by Barton (17) for index fingers, and of the same polarity.

Relation of Polarity and "Handedness." However, four left-handed subjects in the group all averaged negative instead of positive; that is, the right hand was negative to the left one. The finding of a polarity which seems to be related to the "handedness" of the individual parallels in electric terms the temperature observations of Heiser and Cohen (22) who reported that in four out of five right-handed subjects tested by them, the right wrist was warmer than the left one, while in a left-handed person, the converse was true.⁶ In attempting to account for this difference, these authors suggested that it might be due to the "relative dominance of the limbs."

Table 1 shows the difference in polarity of finger potentials between three left-handed and five right-handed individuals. Subject 67 appeared at

⁶ One of us (J. M. S.) had the opportunity of examining more extensive data of Heiser and Cohen which confirmed these findings but were not included in the original report cited.

first to be an exception; that is, although right-handed, she steadily maintained a negative charge. The apparent discrepancy here was later explained by the fact that this woman's duties were largely concerned with pulling and pushing heavy file drawers with the *left* hand. She is therefore included in both groups.

It is impossible for us to explain the constancy of opposite polarity in finger potentials related to handedness if the electric quality of the finger-tips is assumed to be referable to the ovaries.

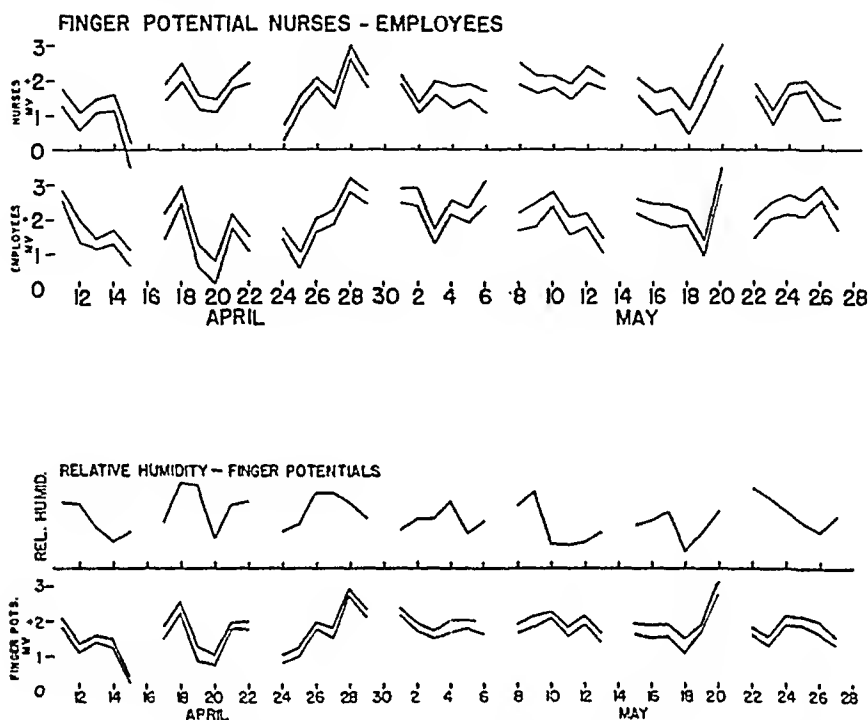


Fig. 3. *Upper curves.* Daily average finger potentials of the nurses and female employees plotted as separate groups. Bands for each group indicate limits of probable potential averages. Potential changes within the bands are not statistically significant, and the curves are in reality indeterminate within the zone. The similarity of the band changes indicates some factor common to both groups which modifies the magnitude of the finger potentials. The sign of the finger potentials obtained from the left-handed subjects listed in table 1 has been changed to a positive value in plotting these curves.

Lower curves. Total combined average daily finger potentials of nurses and employees plotted against the relative humidity. The finger potential curves are subject to the same restrictions as outlined for the upper curves of this figure. Since there are now more observations for each day, the band width is appreciably less.

The Possible Rôle of Environmental Factors in Voltage Changes. When the mean daily finger potentials of all individuals within each of the two groups (group 1, nurses; and group 2, employees) were averaged, and the average of each group was plotted separately, the similarity of the two graphs during some weeks is quite evident (fig. 3, upper curves). The two bands plotted for each group show the maximum and minimum probable variations in potential for a given day. The average potential falls between the two bands, and even though it may be calculated and assigned a mathematical quantity, it is in reality statistically indeterminate within the bands.

Considering these limitations, we find that the potential change of both groups from day to day was in the same direction 86 per cent of the time. Such a relation indicates some common environmental influence acting on both groups. In order to check this hypothesis, several environmental factors were considered: namely, temperature, barometric pressure and relative humidity. Daily measurements taken from the noon observations of the Boston Weather Bureau were utilized. They showed the following percentages of changes in the same direction as the combined group potential averages:

Temperature.....	52 per cent
Barometric pressure.....	55 per cent
Relative humidity.....	66 per cent

These percentages are not large, and in view of the fact that the various subjects worked in different parts of the hospital, the only factor listed which possessed any high order of similarity between groups was the barometric pressure. Obviously neither the outdoor temperature nor any particular indoor temperature would constitute a common factor. Changes in the external relative humidity are usually reflected in the indoor relative humidity, although not necessarily in a one-to-one ratio. The changes in finger temperature with variations in relative humidity are complicated by the fact that the direction of the temperature shifts due to relative humidity fluctuations may change phase depending upon the particular environmental temperature (23). The correlation of average potential values with changes in relative humidity is seen in the lower plot of figure 3.

The high degree of similarity in direction of potential shifts in the two groups of women studied simultaneously pointed to a common environmental influence as a factor underlying the observed fluctuations. Furthermore, the observation that the more active, and possibly, warmer, hand appeared to show a positive polarity with respect to the less active one, suggested the investigation of temperature as a factor in finger-to-finger potentials.

III. THE THERMAL FACTOR IN "FINGER-TO-FINGER POTENTIALS." An experiment was therefore devised whereby the electric potential difference between corresponding fingers of opposite hands (or fingers of the same hand) could be measured while the temperature of the saline solution that served as electrode for one finger was varied slowly (about 1°C. per min.) between the limits of 12° and 46°C.⁷

Procedure. Two one-liter pyrex beakers were filled with physiologic saline solution. The fluid in one beaker was at room temperature, and in the other at some temperature below 10°C. Each beaker was connected by a salt bridge to a Ag-AgCl electrode system in saline solution at room temperature. The Ag-AgCl electrodes in turn were connected to a special Leeds & Northrup Multi-point Micromax Recorder (L. & N. Catalog no. 40356-S-4). This recorder was converted so that a continuous tracing of the potential was made. It was designed to operate over a very wide range of input resistances. Satisfactory records could be obtained from circuits which varied from zero to 250,000 ohms. The chart paper was moved at a uniform rate by means of a synchronous motor-drive system.

⁷ It may be well to point out that finger temperature is not necessarily the same as the environmental temperature, but may easily differ by 10°C. from the latter (23, 24).

The beaker containing the cold salt solution was placed on a 500-watt electric heater. Electric energy was supplied to the heater to increase the temperature of the saline solution at a uniform rate of 1°C. per minute. Since the amount of heat needed changed constantly throughout the experiment, automatic control was required. This was provided by a Leeds & Northrup Model N control instrument. The controller was activated by means of a resistance thermometer placed in the cold salt solution. The controller contacts were capable of carrying the electric heater current without auxiliary relays. The controller was designed to regulate temperature over a range of 0° to 50°C. In order to raise the temperature of the solution at the rate of 1°C. per minute, a General Electric Telechron motor drive was coupled rigidly to the shaft of the control point-setting dial of the L. & N. controller. The synchronous Telechron motor then turned this dial at a rate which increased the temperature of the saline solution 1°C. per minute. The actual error due to thermal lags, etc., was not more than $\pm 0.1^{\circ}\text{C.}$ at any instant. In practice, the temperature of the cold beaker was initially less than 10°C. This was necessary so that the controller might have time to overcome the large thermal lag of the cold heating system. With this precaution in starting, the rate of temperature increase was constant slightly before 12°C.

When the temperature of the saline solution reached 11°C. , the subject dipped the index finger⁸ of the left hand in the beaker of saline solution at room temperature, and the third, fourth and fifth fingers of the same hand, in the cold salt solution. The electrode circuit was in contact with the recorder between 12° and 46°C.

The recordings in the present investigation differ from those obtained in the daily finger-to-finger measurements reported above. In the experiments described here, the beaker of solution to be heated was used as the reference electrode. This was done merely for the sake of convenience, so that the curves, as recorded, read correctly from left to right, and the potential is represented on the chart as increasing in an upward direction.

The fluid in both beakers was automatically stirred vigorously throughout the experiment. Since the chart speed was constant, and the rate of temperature increase was likewise a constant, the resulting curve was automatically traced in the proper rectilinear coordinate system.

*Curves Relating Thermal to Potential Changes.*⁹ Using this procedure, over 200 thermal potential difference (T. P. D.) curves have been recorded. Approximately 100 of these, obtained from daily measurements on the same subject throughout four normal menstrual cycles, show homogeneity within a given cycle, although the character of the curves may change from cycle to cycle. The recordings during the first and fourth cycles appear quite similar. Occasionally the repetition of detail in a curve from day to day is striking. Daily recordings usually show the changes to be progressive, but they may at times be abrupt. Figure 4 illustrates several different types of curves that may be obtained.

The magnitude of potential change with temperature averages approximately 1 mv. per degree Centigrade. However, considerable departure from this average may be observed.

When the temperature was kept relatively constant, as is illustrated in curve A of figure 5, the potential after an interval of 5 minutes maintained a fairly uniform level. The slight slope of the potential curve after the first 5 minutes

⁸ The reason for using the index finger was simply for convenience; any other finger gives the same results.

⁹ The thermal potential difference data have been independently checked at Loomis Laboratory by Dr. Hallowell Davis of the Department of Physiology, Harvard Medical School (25).

was doubtless due to the fact that one of the beakers of saline solution unavoidably warmed up from 24.1° to 25°C . Curve *B* was obtained immediately after curve *A* and shows the finger potential change over the range of 12° to 46°C . It is interesting that when the temperature reached 24.4°C ., the voltage recorded on curve *B* was equal to that on the isothermal curve *A* at the same temperature.

It should be noted that position did not affect the T. P. D. curves; that is, the results were the same, whether the subject was sitting up or resting horizontally on a comfortable bed. A cut on the finger invariably reduced the magnitude

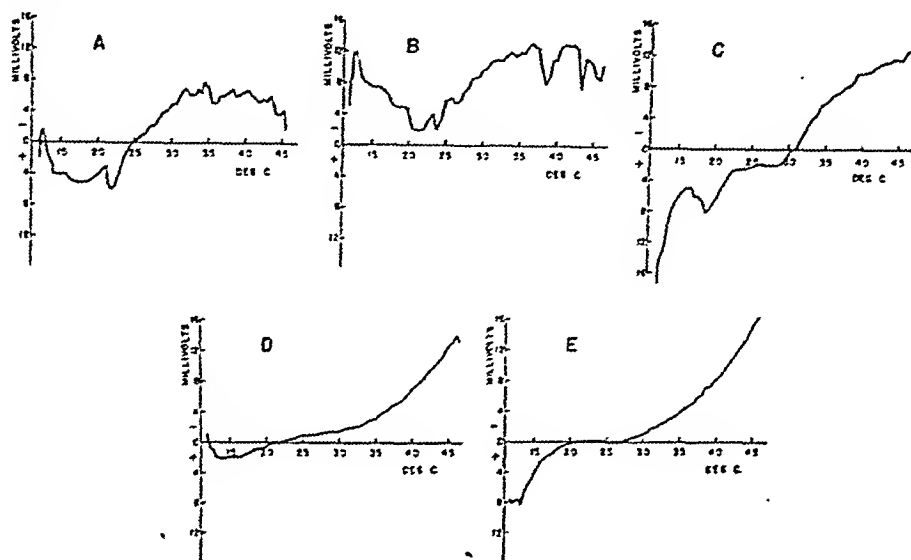


Fig. 4. Thermal potential difference curves illustrating the variety of these. These have been plotted from data recorded by the Leeds & Northrup Micromax instrument before the temperature axis was made uniform. Other T. P. D. curves mentioned in the text, but not shown for lack of space, were plotted automatically on a linear temperature axis by the method described.

Curves *A*, *B*, and *C*, from the young woman who was studied for four normal cycles, were taken at intervals of several days.

Curve *D* was obtained from a young pregnant woman who gave this same type of curve repeatedly.

Curve *E*, recorded from an anovulatory female, represents the general type most frequently obtained from such women and from male subjects.

Curves *D* and *E* should not be regarded as characteristic of the individuals. They are merely those most frequently encountered in the subjects mentioned. Exceptions have been recorded, and as yet there are not enough data to warrant specific conclusions.

of the potential change and at the same time usually caused a large shift in the base-line. The cut also tended to "short circuit" the normal T. P. D. effect.

Thermal Potential Curves During Narcosis. In order to rule out any possible effect on the subject of extraneous stimuli which might influence the nature of the potential changes observed, a curve was recorded $1\frac{1}{2}$ hours after the administration of 3 grains of pentobarbital. The subject was completely asleep and entirely unaware of the experiment in progress. There was relatively little difference between this curve and one obtained with the subject wide awake. Likewise, a recording made while she was under deep ether anesthesia did not differ essentially from curves obtained while she was awake. Therefore, what-

ever the origin of these potentials, it is evident that they are not greatly modified by narcosis or anesthesia.

Reversibility of Thermal Potential Changes. In an effort to ascertain whether or not the mechanism giving rise to these potential differences constitutes a reversible process, the usual curve was recorded as the temperature rose to 46°C., but when that point was reached, the temperature was lowered at the same rate (1°C. per minute) to 12°C. The reversible nature of the thermal potential changes, clearly demonstrated in the right-hand curves of figure 5, is particularly interesting from the standpoint of thermodynamics.

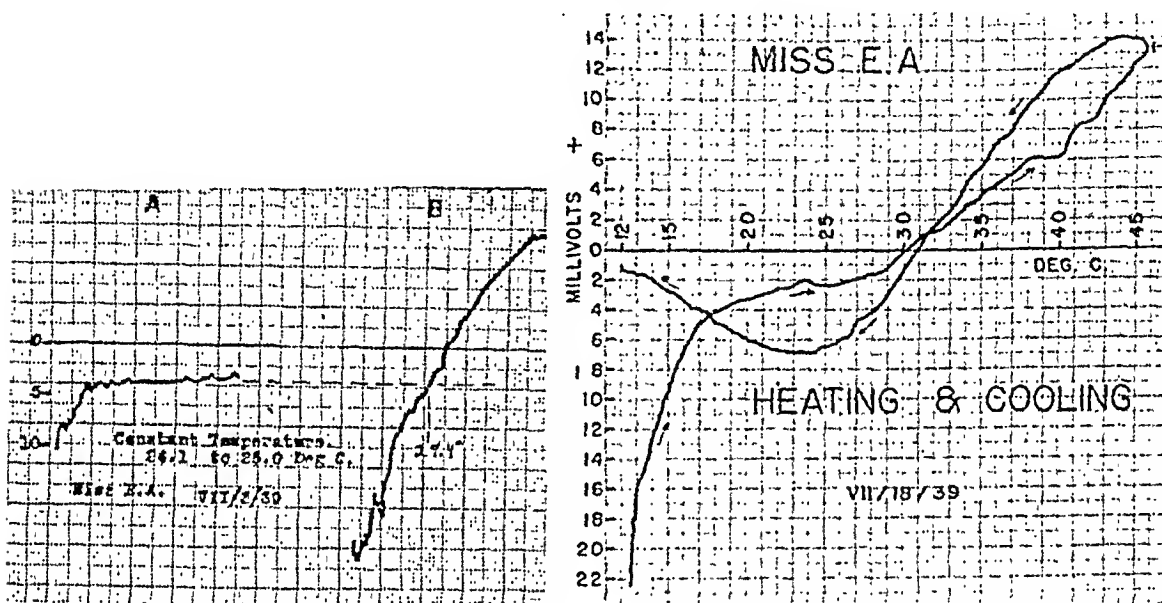


Fig. 5. *Left-hand curves.* Curve A shows the potential obtained under approximately isothermal conditions. Potentials are in millivolts. The potential changes comparatively rapidly during the first 5 minutes and then changes very slowly.

Curve B, taken immediately after curve A, was recorded in the usual way with the temperature increasing 1°C. per minute from 12° to 46°C. Note that the potential at 21.4°C. is the same in both curves.

Right-hand curves. Thermal potential difference curves showing reversibility of process by heating and cooling. The saline solution was warmed at the rate of 1°C. per minute up to 46°C. and then cooled at the rate of 1°C. per minute to 12°C. The arrows indicate the direction of the potential change. The difference between the curves below 23°C. may or may not be significant. Some individuals show a fair degree of similarity between the two curves and others do not, as in this case. Curves plotted from data obtained by the L. & N. Micromax Recorder.

The Sympathetic System and Thermal Potential Changes. It is reasonable to suppose that the general shape of the T. P. D. curves, in regard to both gross and minor features, may be due to nervous control of the vascular system. To check this possibility, curves were recorded from a control and from a sympathetomized rabbit, measurements being made between the ears of each animal.¹⁰

¹⁰ Dr. Philip M. LeCompte and Dr. Hallowell Davis of the Department of Physiology, Harvard Medical School, kindly assisted in the rabbit experiments. Doctor LeCompte carefully sympathetomized the rabbits several days preceding the experiment.

Sympathectomy, performed about six days prior to the experiment, consisted of removal of the superior cervical ganglion and section of the right dorsal and ventral auricular nerves at the base of the ear. The rabbit to be tested, narcotized by an intraperitoneal injection of pentobarbital, was fastened on its back on an animal board, with the head placed so that the ears hung down over the end of the board and into the beakers containing the saline solution. The right ear was immersed to a depth of approximately 7 cm. in the beaker to be heated, and the left ear was similarly dipped in the solution maintained at room temperature.

The change of potential recorded in these curves was about 0.5 mv. per degree Centigrade. The shape of the curve is clearly unaffected by any function of the sympathetic nervous system. This experiment was repeated on the same control rabbit and on a second sympathectomized animal with practically identical results.

The curves obtained from rabbits seem to possess no features which might distinguish them from curves of human subjects.

IV. THE RÔLE OF TEMPERATURE IN ABDOMINOVAGINAL POTENTIALS. In view of the evident influence of temperature on finger-to-finger potentials, it was of interest to ascertain whether or not the same general relations would hold true in abdominovaginal voltage determinations.

Isothermal Contour Patterns. Since the reference electrode employed in measurements of the so-called "abdominovaginal sign" of ovulation had been applied on the suprapubic skin region (1-3, 6, 9-12), it seemed necessary, first of all, to check the degree of temperature variations over skin surfaces. This was accordingly performed over the abdomen and thighs of a human subject. The results are conveniently represented by isothermal contours (fig. 6).

Procedure. The temperature was measured by means of a small thermocouple of no. 42 B. & S. gauge copper and constantan wire. The exploring thermal junction was mounted on the surface of a piece of cork 2 mm. square, which in turn was fastened to a small glass tube. The exploring junction had a very small thermal capacity and reached equilibrium with the skin in a fraction of a second.

The reference junction was immersed in a water bath at approximately room temperature inside a vacuum Thermos flask. This gave sufficient stability to the temperature of the reference junction, since there was at most a differential of one or two degrees between the reference junction water bath and room temperature.

The thermocouple system was calibrated, each time it was used, by placing the exploring junction in a water bath containing a standard mercury thermometer graduated in steps of 0.02°C . The water bath was then warmed over the range of skin temperature previously measured. A Leeds & Northrup (Catalog no. 2500-C) Type R galvanometer was used as a null-indicating instrument.¹¹ Even with the relatively long natural period of 5 seconds, it was easy to obtain sufficiently accurate temperature determinations in 3 to 4 seconds. The short time is made possible by anticipating the deflections of the galvanometer and rapidly manipulating the potentiometer to keep the deflection at the zero point.

Results. The isothermal contour patterns (fig. 6) closely resemble the isopotential curves, as recorded in figure 1, and show clearly how the temperature

¹¹ This was not the proper galvanometer for the purpose, but was the best available at the time.

varies over the particular surface areas investigated. By checking the contour lines it is readily seen that points only 4 or 5 cm. apart may differ in temperature as much as 2° to 3°C . As regards stability, the isothermal and isopotential patterns are similar.

Benedict, Miles and Johnson (26) have published temperature curves obtained by moving a thermocouple down the nipple line of a human subject. Similar curves may be derived from figure 6 by plotting the temperature values along a straight edge placed parallel to the nipple line.

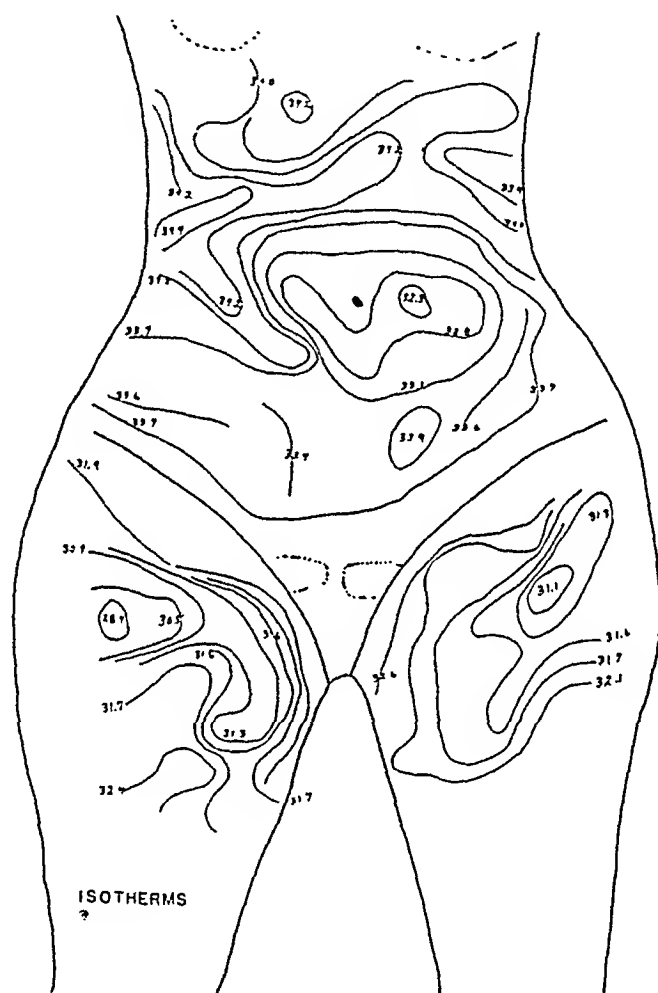


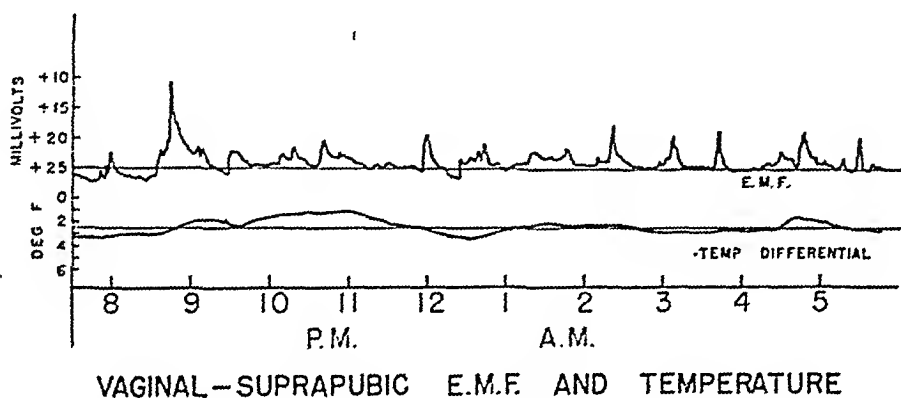
Fig. 6. Isothermal contours measured on a female subject. The isothermal contours exhibit the same type of "high" and "lows" as do the isopotential patterns; cf. figure 1. The temperature range of the isotherms shown in figure 6 is from a "low" of 28.4° to a "high" of 34.4°C ., a difference of 6.0°C :

Thermal and Potential Abdominovaginal Gradients. Figure 7 is a traced composite of a portion of the electric and thermal record obtained simultaneously when a vaginal and a suprapubic electrode containing thermocouples were employed. Such an experiment is interesting in view of the report of Burr and Barton (27) in so far as it furnishes data on the variation of another parameter: namely, temperature, which is known to fluctuate during sleep.

Procedure. The electrodes were agar blocks made with physiologic saline solution, agar and Arlex.¹² The electrode proper consisted of a chlorided silver plate immersed in the agar mass before the latter solidified. Small iron-constantan thermocouples inserted in thin-walled glass capillary tubes were also embedded in the agar mass. The thermocouples were connected so that each agar block electrode contained two thermal junctions to increase the temperature sensitivity.

The output of the thermocouples was connected to a Leeds & Northrup Micro-max Recorder. The potential leads were connected to another Leeds & Northrup Recorder. The L. & N. Recorders had the same paper speeds and each was operated by a synchronous motor.

Results. Unfortunately the agar electrode mass has a relatively large thermal capacity, as well as poor heat conductivity. The temperature record is therefore greatly smoothed and will tend to lag considerably in time. However, even in spite of these difficulties, it is reasonably clear from figure 7 that the



VAGINAL—SUPRAPUBIC E.M.F. AND TEMPERATURE

Fig. 7. Composite of simultaneous recordings of vaginal-suprapubic potential and temperature differences. These were automatically traced on two separate Leeds & Northrup Micromax Recorders. Each record was traced and the composite carefully put together to insure simultaneity. There is quite an appreciable thermal lag, as recorded, due to the poor heat conductivity of the agar mass, as well as its relatively large heat capacity. The temperature record may be seen to follow the base-line of a greatly smoothed electric record.

mean changes in temperature follow the potential changes. Most of this record shows the electric and thermal tracings to be in phase; that is, an increase in temperature is accompanied by a rise in potential. Frequently, however, the phase relations may shift for several hours; i.e., an increase in temperature now is accompanied by a *decrease* in potential. These potential-temperature phase reversals are entirely consistent with the results obtained in the thermal potential difference experiments. As shown in curve A of figure 4, the potential may not increase uniformly over the temperature range of 28° to 34°C., the same temperature range as represented in the isothermal plot of figure 6. In curve A of figure 4, the potential is seen to increase with temperature from 28° to 31.8°C., and then it proceeds to fall 1 mv. between 31.8° and 32.5°C., even though the temperature is still rising.

¹² Arlex: Trade name of commercial sorbitol, an excellent "humectant", manufactured by the Atlas Powder Company, Wilmington, Delaware.

It should be emphasized that both the thermal and electric curves of figure 7 represent differential values; that is, the temperature or electric *difference* between the vaginal and suprapubic electrodes.

V. pH AND POTENTIAL CHANGES. Since the experiments described above clearly indicated a relation between temperature and bioelectric potentials, the next consideration was the effective mechanism.

Evidence that the thermal factor might act through its influence on pH was suggested by the data of Sribyatta and Bazett (28) on decrease of pH of blood in vivo with increase in temperature. Through a temperature range (10° – 45° C.) approximately the same as that within which the T. P. D. curves of the present study were recorded, these workers obtained a difference of 0.36 pH. Now, if one assumes that the thermal skin temperatures are due to a pH concentration-cell system made up of blood, tissue fluids and the skin, and since a change of 1 pH unit is equal to a potential shift of about 60 mv., a decrease of 0.36 pH would involve an increase of approximately 22 mv. This figure is of the same order of magnitude as the voltage changes recorded in the T. P. D. curves over a corresponding temperature range. The pH values, as plotted by Sribyatta and Bazett, and the T. P. D. curve C of figure 4 are similar.

Stasis and Potential Changes. In view of this agreement between calculated and observed values of E. M. F. when thermal skin potentials were assumed to be due to a pH concentration-cell system, we next undertook to determine if one could obtain significant changes in potential by employing some means to affect pH. An experiment was therefore devised whereby the temperature was kept constant, but a shift in blood and tissue pH was induced by arterial and venous stasis.

The same set-up was used as previously in obtaining the T. P. D. curves represented in figure 4, but in this case the fingers of the experimental hand were placed in a beaker of saline solution kept at a constant temperature of 40° C. $\pm 0.1^{\circ}$ C., while the reference or contralateral fingers were immersed in salt solution maintained at approximately 23.2° C. A Riva-Rocci cuff was placed on the experimental arm as for determinations of blood pressure.

For a period of 20 minutes normal conditions prevailed. Then the cuff was rapidly inflated to 40 mm. Hg above systolic pressure. Following a brief lag, the potential rose to a plateau (fig. 8). After 10 minutes, during which pain was experienced and the arm became quite cyanotic, "Bier" spots being clearly visible near the end of this period, the pressure in the cuff was rapidly released, whereupon the potential fell steeply. There was, however, a lag of 10 to 15 seconds between the release of the pressure and the beginning of the potential drop. The potential decreased rapidly and seemed to overshoot—possibly because of excess blood flow due to the relaxed condition of the capillaries under the high concentration of CO_2 which had been built up during the period of arterial and venous stasis.

When the cuff was again inflated 10 minutes later, the same phenomenon was observed. In the last part of the record, a similar potential rise was induced by warming the experimental hand.

This particular experiment may be repeated as often as desired with substantially identical results.

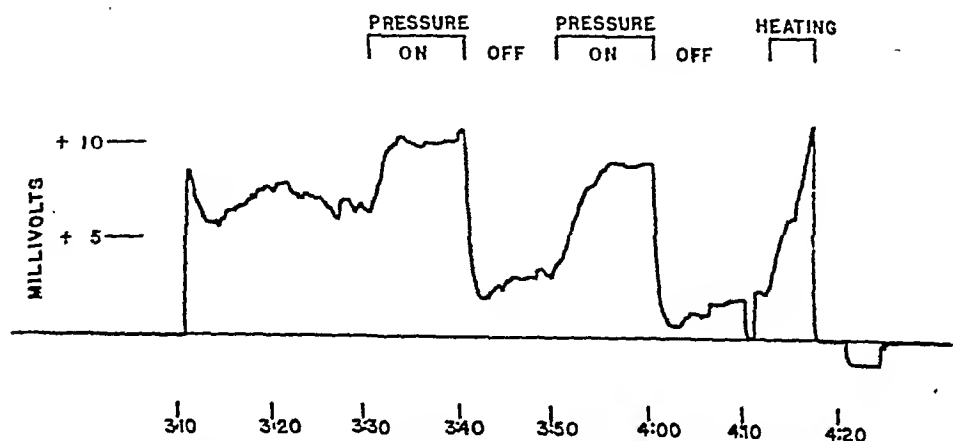


Fig. 8. Finger-to-finger potential changes induced by arterial and venous stasis by means of a Riva-Rocci cuff on one arm.

When the pressure was released at 3:40 p.m., there was a sudden drop of 8.8 mv. in the potential difference between the control and the experimental arm. From data compiled by Peters and Van Slyke (29), a difference of approximately 0.14 pH may be expected in human blood between the condition of rest and that of heavy exertion. This corresponds to a voltage shift of 8.7 mv., a figure in close agreement with our result.

The last part of the record marked "heating", and beginning at about 4:11 p.m., shows the potential change on warming from 40° to 46°C. The break in the curve is caused by the momentary cessation of the heating at 43°C.

Curve reproduced photographically from untraced original.

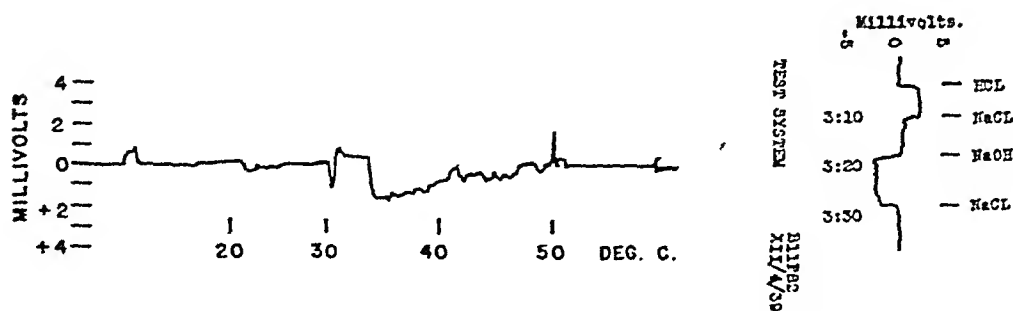


Fig. 9. *Left-hand curve.* Thermal potential differences induced by heating heparinized whole blood in one arm of a "U"-tube, the ends of which were covered with a semipermeable membrane. Little potential change appeared before 30°C. At 50°C. the potential fell to zero and no further potential change was observed, even though the heating was continued to 60°C. It is quite possible that the 50°C. point may be significant in this connection, as most of the proteins would be completely denatured at or before this temperature was reached. The curve is an untraced photographic reproduction.

Right-hand curve. Potentials recorded in test system when dilute HCl and dilute NaOH are added on one side of the semipermeable membrane. Curve traced from original recording.

VI. EFFECTS OF TEMPERATURE AND pH POTENTIAL IN VITRO. In order to study directly the effect of thermal and pH changes on potential, experiments were performed on a synthetic system.

1. *Temperature.* The set-up consisted of a "U"-tube containing heparinized whole venous blood; across the ends of the "U"-tube was stretched a semipermeable membrane. One arm of the "U"-tube was inserted in the beaker of saline solution serving as the reference terminal. The other arm of the "U"-tube was dipped in the salt solution to be heated. The curve obtained, as the saline solution was heated, is shown in figure 9 (left-hand plot).

2. *pH.* The "U"-tube used above was modified by making a glass "T" connection at the vertex of the inverted "U" and inserting a very small bore rubber tube down the "T" and along one arm of the "U". The side having the rubber tube was called, for convenience, the "experimental arm." In the set-up as described above, but with the "U"-tube filled with the same saline solution as in the beakers, the only potential in the system was the small electrode potential.

When dilute HCl in saline solution was run through the rubber tube to the "experimental arm," a potential change in the positive direction was observed, as shown in the right-hand curve of figure 9. When pure saline solution was substituted, the potential returned to the initial value. The introduction of dilute NaOH in salt solution, on the other hand, was followed by voltage changes in the opposite direction.

The direction of the potential change elicited upon introducing dilute HCl into the system was the same as that caused by the acidosis resulting from the arterial and venous stasis produced in the experiment described above. The results of the in vitro experiments therefore substantiate those obtained in vivo, showing clearly a definite relation between thermal and pH factors and potential.

DISCUSSION. The evidence presented in figure 1 indicates the great difficulty of locating on the skin surface a stable reference electrode. The potential gradient is occasionally so steep that a difference of 22 mv., or more, may be found between two points no further apart than 4 cm. This difficulty in maintaining the stability of a reference electrode on the skin surface offers in itself a rational explanation for the inconsistent results previously obtained in connection with the so-called "abdominovaginal sign" of ovulation.

The rôle of emotional factors in relation to stability of isopotential contour patterns brings up an interesting point with regard to the findings of Altmann (8) on the sow. Since Altmann's potential values, as published, represent the averages of several daily observations taken at various times during the cycle, it is not surprising that the voltage differences at ovulation time are higher than those at diestrus, for she herself comments on the "frequent and typical grunting, mounting, and restlessness" of the sow during heat. She records that in the course of estrus, spontaneous activity increased up to about twice the non-estrous level.

Inasmuch as in our experiments on finger-to-finger potentials we were unable to duplicate the results of other workers who reported cyclic electric changes which they correlated with ovulation time, it may be pertinent to remark on certain aspects of these studies. In regard to the findings recorded by Barton (17, 18), it should be pointed out that the method of reducing all cycles to deciles grossly ignores any possible biologic connection between ovulation and menstruation. While the method is admittedly statistically convenient, it may not legitimately be applied where there exists any causal relation between a series of variables observed over different intervals of time, and especially when there is a functional dependent relation between the separate observations.

In connection with the experiments of Altmann et al. (19), it should be noted that since the cycle lengths of their subjects ranged from 23 to 61 days, the results are hardly applicable to the normal cycle. Furthermore, the temperature and vaginal smear data, collected for the purpose of furnishing correlative and supporting evidence as to the time of ovulation, were admitted by the authors to be not entirely satisfactory. The temperature records, they state, did not always represent basal values. The vaginal smears, stained by the original technic of Papanicolaou, which is now considered quite inadequate, manifested a "rather weak expression of the phases of the cycle." The latter was attributed to the fact that the subjects were college women.

The temperature potential difference (T. P. D.) curves recorded in this study clearly demonstrate a definite relation between thermal and electric changes, and indicate that the mechanism by which temperature influences potentials is concerned, at least in part, with changes in pH. Certain non-linear results obtained in the course of our experiments bring to mind the investigations of Loeb (30) on the effect of hydrogen-ion concentration of protein solutions in modifying the potentials across membranes.

On the basis of the asymmetric temperature differences noted by Heiser and Cohen (22), as well as the finger potential data presented above, it would seem highly probable that there is a small but significant pH difference between the venous blood taken from the right and left arms. In right-handed individuals, the pH of the blood should theoretically be slightly lower in the right arm than in the left one.

It may be pointed out that changes of blood and tissue pH with respect to temperature have been little investigated. Measurements of pH have usually been made under carefully standardized temperature conditions, and comparatively meager information is available relating to continuous functions of temperature and pH. The influence of environmental factors on blood pH was investigated by Berg and his associates (31) who found a high statistical correlation between the blood pH of dogs and that of human beings studied over the same period of time. The relation between pH and temperature was less marked than that between pH and barometric pressure. In our own study on finger potentials made simultaneously on two groups of women, very little correlation could be shown between the observed potentials and barometric pressure, whereas a definite relation was noted between potentials and relative humidity. This is probably due to the fact that the action of the pressure change is symmetrical and does not have as large an asymmetric modifying factor as does the relative humidity. The relative humidity may be considered as an asymmetric modifying factor, since there exists an initial difference in temperature between the right and left hands and any change in the relative humidity modifies the relative rates of heat radiation of the two hands.

In attempting to explain the marked E. M. F. changes that have been associated with ovulation, one should take into account the experiments of Mittelman and Wolff (24) who reported great variability in finger temperatures with different emotional states. *Since skin temperature fluctuations are known to be induced by vasomotor and hormonal changes, it seems quite probable that the so-called "ovulation potentials" previously reported, whether elicited between a vaginal and a suprapubic electrode (1-6, 9-12), between two surface areas—abdominal or flank (7, 8, 12), or between fingers (14-19), are measurements only of local changes in*

peripheral cutaneous blood flow, due to alterations in capillary tone. Hormonal changes, which would of necessity be general and diffuse, might easily lead to increased instability of capillary tone, giving rise to fairly large thermal and potential shifts. On this basis, one may perhaps explain the difficulty encountered in attempting to plot either isopotential or isothermal contours on individuals of the so-called "sthenic" type. When one considers the profound vascular and thermal changes which occur with estrus, Rogers' (6) experiments on rats seem to fall readily in line with the results in human beings and rabbits.

In regard to the contention of Altmann et al. (19) that potential and temperature differences between index finger-tips were unrelated, it should be pointed out that no definite conclusion can be drawn from their experiments, for, whereas the thermocouple measures the temperature of a decidedly restricted area in physical contact with its elements, the electric measurements, on the other hand, represent the bioelectric conditions existing over a fairly large area (the surface of the finger-tip), and would not be expected to be related to a point determination of temperature.

Mention should here be made of a recent report by Langman and Burr (32) of a negative shift in potential difference between the cervix *uteri* and the ankle noted at certain times in the menstrual cycle, whereas tests made on other days of the cycle showed the cervix to be positive to the ankle. Negativity was observed during the menses, as well as at various times in the intermenstruum. Because in two cases artificial insemination performed during the negative phase resulted in pregnancies, the authors regard the negative shift of potential of the cervix *uteri* as a sign of ovulation.

In this connection it seems pertinent to suggest the possibility that the potential change recorded by Langman and Burr may be dependent on cyclic variations in the pH of the cervical mucus. Pierra (33), reporting an increase in alkalinity of cervical mucus in the intermenstruum, stated that while the rise in pH could not be considered a definite test of ovulation, it might help to determine when a woman was especially fertile. Later, Lamar, Shettles and Delfs (34) noted increased alkalinity of cervical fluid during the menses, as well as in mid-cycle, and observed that this change was accompanied by a rise in the amount of secretion together with increased penetrability to spermatozoa, as well as longer survival time of the latter. The possible rôle of cervical pH in accounting for unexplained causes of sterility has been suggested by Schockaert and Delrue (35).

May not the increased negativity of the cervix during catamenia and in the intermenstruum, recorded by Langman and Burr, be merely a measure of increased alkalinity of cervical secretion at these times? Such a process can be duplicated by the *in vitro* experiment described above (fig. 9, right-hand curve) in which a shift of potential difference in the negative direction took place when NaOH was substituted for NaCl in the synthetic system. On this basis, then, one would expect the negative phase to be the logical time for artificial insemination to succeed, due to the increase of alkalinity of the cervical mucus associated with optimum conditions for penetration and longevity of spermatozoa. Strictly speaking, then, the negative shift in potential between the cervix *uteri* and the ankle, reported by Langman and Burr, may be regarded not as a test for

ovulation, but as an indication that conditions in the cervix are favorable for the survival and successful functioning of spermatozoa.

SUMMARY AND CONCLUSIONS

1. By plotting isopotential contour patterns on human skin areas, it was difficult to locate properly on the surface a reference electrode which would be stable in potential. This instability, which may be in part, at least, related to emotional factors, made it impossible in our cases to obtain isopotential contours on preoperative patients near ovulation time. With the broad ligament as a reference electrode, direct measurements on ovaries *in situ*, during laparotomy within the estimated ovulation time, failed to show any marked potential gradients. The difficulty of maintaining the stability of a reference electrode on the skin surface offers in itself a rational explanation for the inconsistent results previously obtained in connection with the so-called "abdominovaginal sign" of ovulation.

2. When adequate precautions were taken to rule out spurious readings, no marked changes could be demonstrated in daily finger-to-finger potentials of two groups of female subjects studied for about nine consecutive weeks. In women with normal menstrual habits, there was no evidence of cyclic potential changes between finger-tips that might be related to the occurrence of ovulation. The index fingers did not appear to possess any unique property when voltage readings obtained from them were compared with determinations made on other pairs of fingers. The observation that polarity was associated with relative activity (and possibly temperature) of one hand as against the other, as well as the high degree of similarity in direction of potential shifts of the two groups of women studied simultaneously, pointed to a common environmental influence as a factor underlying the observed fluctuations, and suggested the investigation of temperature in relation to finger potentials.

3. Temperature was shown to influence bioelectric potentials, the effect consisting, as a rule, of an increase in potential of the warmer area in a positive direction with respect to the cooler one. The relation of skin potential to the surface temperature was found to be reasonably stable and reproducible within certain limits over the range from 12° to 46°C. The change of potential with temperature was reversible; it was not affected by the sex, state of consciousness, or position of the subject. The results were similar whether fingers of the same or of opposite hands were tested, and, as shown by the rabbit experiments, were apparently not dependent on sympathetic innervation of the areas studied.

4. The evidence presented suggests that the mechanism through which temperature affects potential may be bound up with changes in pH. If one assumes the thermal skin potentials to be due to a pH concentration-cell system and, on the basis of previous experiments relating temperature and pH, one calculates the values of E. M. F., the theoretical and observed figures agree closely.

5. Bioelectric phenomena previously reported as related to ovulation are due primarily to cutaneous vascular conditions and possibly only remotely and to a

slight degree to ovarian influence. Local changes in blood flow affect the focal temperature, which in turn reacts on the focal pH, the fluctuations of which directly determine potential variations. Because the hormonal factor is only one of several affecting vascular phenomena, it is our belief that potential changes recorded thus far cannot be attributed solely to ovarian activity, and that therefore the electric methods hitherto proposed are unsuitable for the detection of ovulation.

We are greatly indebted to Dr. Hallowell Davis for valuable suggestions during the course of these investigations.

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RÔLE OF THE KIDNEYS IN THE RESISTANCE OF RATS TO HEMORRHAGE

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Recent work has suggested that the kidney may play a rôle in resisting shock due to hemorrhage. This concept is supported by the following observations: 1, renal venous blood obtained from dogs which have been bled causes a greater increase in blood pressure than does similar blood from normal animals when injected into a recipient nephrectomized dog (1); 2, hemorrhage in nephrectomized dogs results in a lower blood pressure and a higher percentage of deaths than the same amount of blood loss in intact control animals (1); 3, in adrenalectomized dogs the renal mechanism seems essential for pressor activity (1); 4, blood from animals after hemorrhage, as tested on guinea-pig ileum, shows an increased content of angiotonin and a decreased content of renin-activator, as compared with a marked increase in renin-activator content in adrenalectomized-nephrectomized dogs (2); 5, blood pressure can be restored in dogs made hypotensive from hemorrhage by injection of renin-activator, presumably because secretion of renin after hemorrhage results in an exhaustion of renin-activator (3). The experiments here reported offer evidence that in the rat—as in the dog—the kidneys play a rôle in maintenance of blood pressure and prolongation of life in hypotension due to hemorrhage.

METHOD. In each experiment a pair of rats of the same sex and of approximately equal weight and age were used. These were given as nearly as possible identical treatment except that in the test animal the renal circulation was severed from its connection with the general circulation, while that of the control animal was left intact. A cannula was inserted into the abdominal aorta just proximal to its bifurcation and blood pressure readings were taken with a mercury manometer connected directly to the cannula. In five experiments the rats were anesthetized with 1 per cent sodium pentobarbital given intraperitoneally and later supplemented by intra-aortal injections of smaller quantities as necessary. In the other two experiments ether was used until approximately 0.5 cc. of 20 per cent paraldehyde in saline could be injected intra-aortally. Heparin in saline was administered to prevent clotting. Blood was withdrawn and injections made through a three-way tap connected to the aortic cannula.

Every effort was made to control the effects of such trauma as was necessary in order to carry out the experiments. Thus, in two experiments complete bilateral nephrectomy was performed through the abdominal cavity in the test animal, and renal decapsulation with as nearly as possible the same amount of trauma was performed in the control animal. In one experiment the kidneys of both animals were freed from their capsule and peritoneal covering and ligatures

which had been passed around the pedicles were tied in the test animal and left loose in the control. In the remaining four experiments ligatures were passed around the vessels of both kidneys with a curved needle, avoiding trauma to the kidneys, and these were tied in the test animal and left loose in the control. The average duration of the operation was 20 minutes, so that there was relatively little difference in the duration of shock in the two animals, but in addition the order of operation on test and control rats was alternated in successive experiments. The technique of bleeding varied according to the amount required to give a moderate hypotension, but 0.5 cc. portions were removed every 10 minutes until roughly one per cent of total body weight was withdrawn and subsequently blood was withdrawn in 0.25 cc. portions until the animals died.

In one additional experiment adrenalectomy was performed through the abdomen in both test and control animals previous to the above-described operation and bleeding.

As a control to determine the effects of the anesthetic and operation alone two additional experiments were performed. A single rat under sodium pentobarbital anesthesia, without renal circulation, was allowed to remain without blood loss until death. In the second experiment a test and control animal, both under ether and paraldehyde, were allowed to remain similarly until death.

RESULTS. The effects of blocking the renal circulation were striking in that in all experiments the control animal maintained a higher blood pressure after bleeding than did the test rat. This was true also in those experiments in which the initial blood pressure before bleeding was lower in the control than in the test animal. In all but one experiment the control rat lived longer (fig. 1 a-c), and in the single experiment in which the control animal died sooner, its blood pressure throughout the experiment, except for the last eleven minutes, was significantly higher than that of the test animal.

Results of the adrenalectomy were inconclusive since the first blood pressure readings indicated that the operation itself was extremely shocking, but the difference between test and control animals seen in the seven previous experiments were exaggerated in the adrenalectomized pair of rats. Both animals sustained hemorrhage poorly, that without renal circulation markedly less well (fig. 1, d). (In no experiment except that with adrenalectomy could any disturbance of the adrenals or their blood supply be demonstrated on gross post-mortem examination.)

In the control experiments without hemorrhage the one animal under sodium pentobarbital without renal circulation maintained a blood pressure above 90 mm. Hg for $4\frac{1}{2}$ hours. In the two animals under paraldehyde the animal without renal circulation maintained a pressure above 95 for 3 hours, while the control had a consistently lower blood pressure but maintained it above 80 for $2\frac{2}{3}$ hours. This period was longer than the duration of hemorrhage and hypotension, from initial hemorrhage until death, in any of the experiments.

DISCUSSION. Recent work has shown that the kidneys produce a pressor substance following hemorrhage which can be demonstrated by injection into dogs or by *in vitro* tests on guinea-pig ileum, and that nephrectomized dogs main-

tain a lower blood pressure than intact animals (1) (2). The experiments here reported show that rats deprived of their renal circulation withstand hemorrhage much more poorly than do control rats.

The following facts were considered to rule out other causes of the difference in response of test and control animals than the failure of the test animal to secrete a renal pressor substance: The effect of accumulated sodium pentobarbital, which is excreted mainly through the kidneys, was eliminated by experiments in

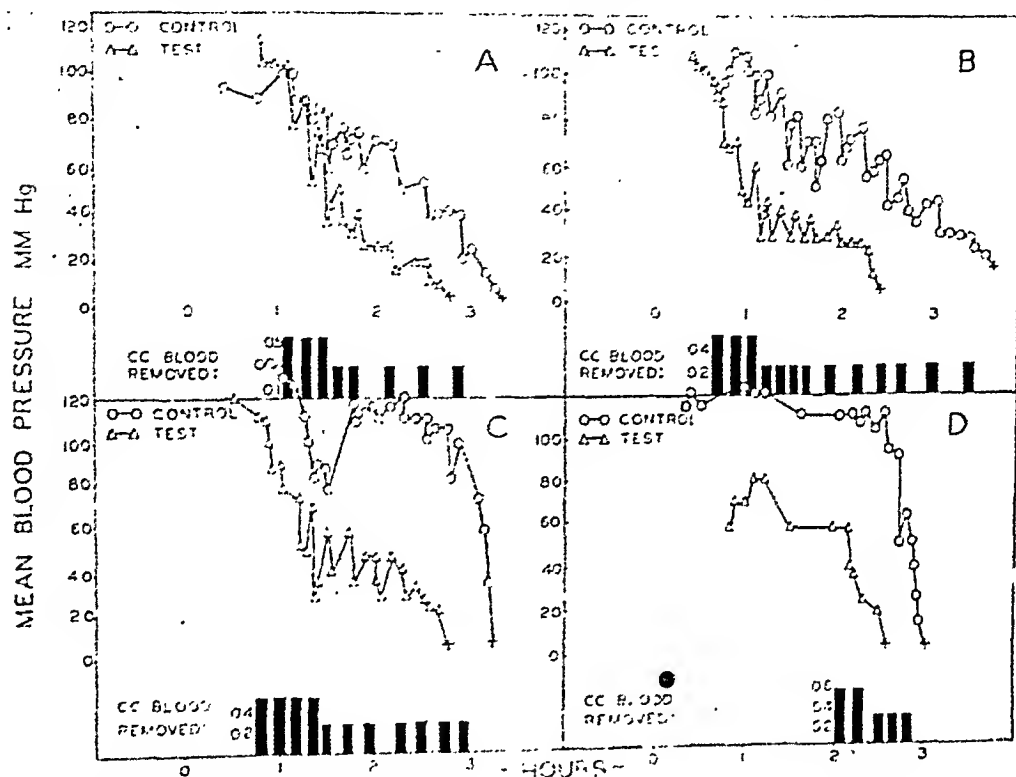


Fig. 1. Effect of hemorrhage on the blood pressure of rats deprived of their renal circulation and on control intact rats.

Fig. 1, a, b. Test animal had bilateral nephrectomy; control animal had renal decapsulation and sham nephrectomy. Both animals under nembutal.

Fig. 1, c. Ligatures passed around renal pedicle of both rats with a curved needle, tied in the test rat and left loose in the control. Both animals under nembutal.

Fig. 1, d. Both animals adrenalectomized. Ligatures passed around renal pedicles of both animals, tied in test animal and left loose in control. Both animals under ether followed by paraldehyde.

which ether and paraldehyde, which are eliminated mainly by the lungs (4), were employed as anesthetics. That the results were due to trauma or removal of the adrenals from the circulation was not considered tenable because of the separateness of the kidneys and adrenals in rats, the failure to demonstrate a damaged blood supply or damaged gland at post-mortem, the much greater shock caused by adrenalectomy than by nephrectomy, and by the fact that the action of the adrenal and kidney seemed to be additive in that adrenalectomized rats without renal circulation withstood hemorrhage extremely poorly as compared with the

control adrenalectomized rat. Although in these experiments no attempt was made to rule out reflexes arising from the kidney as a cause for the results, Hamilton and Collins have shown that the production of a pressor material does not depend entirely on connections with the central nervous system (1).

These experiments do not prove that the renin—renin-activator system, which has been shown to undergo changes during hemorrhage and shock, is responsible for the maintenance of blood pressure in this condition, but such seems the most logical explanation at the present time of the fact that animals deprived of their renal circulation withstand hemorrhage much less well than control intact animals.

SUMMARY

Rats anesthetized with sodium pentobarbital were subjected to repeated hemorrhage, the kidneys being removed from the circulation in one-half of the animals and being intact in the remainder. The nephrectomized animals displayed a greater drop in blood pressure, and died following the removal of a smaller amount of blood than the controls. The effects were not due to trauma to the adrenals because they were not modified when the kidneys of the control animals were manipulated to a degree equal to that required for a nephrectomy. Likewise, the results were not due to failure of excretion of the anesthetic agent because similar effects were observed in animals anesthetized with ether and paraldehyde. The observations support the concept, advanced by others, that the renal pressor mechanism may play a rôle in resistance to hemorrhage and other states attended by acute lowering of the blood pressure.

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THE MEASUREMENT OF BLEEDING VOLUME IN THE DOG FOR STUDIES ON BLOOD SUBSTITUTES¹

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That the response to withdrawal of small volumes of blood changes with the state of the animal and with the volume and composition of the circulating fluid is a matter of common observation. There have been occasional attempts to use this as a means of gauging the severity of cardiovascular damage (Ebert, Stead, Warren and Watts, 1942) or the adequacy of blood substitutes (Gordon, Hoge and Lawson, 1942; Levinson, Weston, Janota and Necheles, 1943). The significance of such studies is obscure, since there has been no systematic investigation of the factors which limit the volume of blood which can be withdrawn from an animal at any particular circulatory level, and since the limiting factors cannot be predicted on *a priori* grounds.

It may be assumed with some assurance, however, that the volume obtained when an animal is bled to death depends upon the volume and the composition of the blood at the start of the bleeding; upon the amount of extravascular fluid which can be brought in, and the efficiency of mechanisms which maintain flow through vital organs, during the bleeding; and upon the ability of vital organs to survive reduction of blood flow toward the end of the bleeding. It appears likely that the relative importance of these factors will vary with the rate of bleeding. Rigorous control of bleeding rate is therefore essential if any interpretation of results is to be attempted. The term bleeding volume will be used in this report, unless it is qualified, to mean the volume of blood obtained by bleeding to death at controlled rates of blood withdrawal.

Comparison of bleeding volumes in groups of differently treated animals might be expected to yield useful information, but the value of the information gained would hardly justify either the labor or the large expenditure of animals which would be required. Data on the factors which govern bleeding volume could be obtained easily and economically if it were possible to repeat the measurement in the same animal at appreciable time intervals. Such repeated measurements are possible if a reliable end-point for the first exsanguination can be found, which will permit extrapolation to death with a fair amount of accuracy. It is obvious that even small blood withdrawals for the production of such an end-point may produce persistent changes in bleeding volume which will alter the value obtained in the second measurement. If irreparable damage is done to the cardiovascular system or its contents on the first withdrawal, reinjection of the drawn blood should yield low second values. If, on the contrary, there is a net gain of circulating fluid during the first withdrawal, and this fluid remains available, second volumes should be higher than the first.

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The studies reported here were undertaken in an attempt to find conditions under which bleeding volume could be determined twice in the same animal, the animal being killed by bleeding on the second test. Since the immediate object was the development of tests for the empirical comparison of blood substitutes (Lawson and Rehm, 1943), conditions were sought under which the second volume would be nearly the same as the first if the blood drawn for the first test was reinjected, and would be very low if no replacement was made. Similar considerations influenced the choice of a 4-hour interval between the first and the second measurement.

METHODS. Dogs kept without food and water for 18 to 24 hours were anesthetized by a single intravenous injection of sodium barbital, 250 mgm./kgm. A tracheal cannula was inserted, a carotid artery cannulated for recording mean arterial pressure, a femoral artery cannulated for drawing blood, and the ipsilateral femoral vein prepared for infusions. Animals were not selected for size, sex, or condition, except that no obviously sick, immature, or pregnant animals were used. About one-fourth of the experiments were done with 0.9 per cent NaCl solution in the carotid cannula and blood-pressure line, with $\frac{1}{2}$ cc. 5 per cent chlorazol fast pink solution injected into the cannula as anticoagulant, the remainder with 5 per cent sodium citrate solution. No difference was observed in the results. The animals were kept in a supine position throughout the period of observation.

Blood was drawn by free arterial hemorrhage in unit volumes equal always to 2 cc./kgm. body weight at intervals of 1 to 4 minutes, the interval between withdrawals being kept constant throughout an experiment. The withdrawal of this volume usually required 5 to 10 seconds, except on the last few bleedings before death, which sometimes required 45 seconds or longer. The relatively long period in each interval during which no blood was flowing was utilized for flushing out the cannula to prevent clotting. The arterial pressure recorded as resulting from a unit volume withdrawal was read at the end of the interval, i.e., just before beginning the next withdrawal.

Heparinization of the drawn blood for reinjection was done by placing in the bleeding cannula before each unit withdrawal enough heparin solution to treat the volume to be drawn. The heparinized blood was filtered through cheesecloth just before infusion. Infusions were started immediately at the end of the interval in which the pre-arranged end-point was reached. No careful control of infusion rate was attempted, the blood being run in rapidly at first until arterial pressure began to rise, then more slowly, the whole infusion being completed within 3 to 8 minutes.

Of the several criteria considered for determining the exact time of death, the most satisfactory was found to be persistent depression of arterial pressure below 10 mm. Hg. To ensure uniformity in handling temporary depressions below this level, it was decided not to begin a bleeding if pressure at the time was below 10 mm., but not to discontinue a bleeding which had been started for any other reason than inability to obtain blood. Fractional withdrawals were thus obtained only on the last bleeding. They were counted as complete

if more than half the required volume was drawn, and not counted if less than half was obtained. The volumes reported are for this reason always multiples of 2 cc./kgm., and are accurate within 1 cc./kgm.

RESULTS. *Preliminary bleeding.* Exploratory studies made it apparent that the conditions sought for the comparison of blood substitutes could not readily be obtained by a simple double-hemorrhage procedure. If the first bleeding was terminated when death seemed imminent, and no replacement was made, arterial pressure not infrequently recovered, and fairly large second bleeding volumes were obtained 4 hours later. Replacement with saline after the first bleeding rather consistently yielded large second bleeding volumes. It was found, however, that neither spontaneous nor saline-assisted recovery of bleeding volume occurred following the second hemorrhage, if it was stopped short of death. Third bleeding volumes under these conditions were consistently and strikingly reduced, many of the animals dying within the 4 hours.

Since comparison of the first and the second volume did not appear to be useful for the present purposes, it was decided to use the first bleeding as a part of the preparation of the animals, and actually to compare the volumes obtained on the second and the third bleeding. To reduce the tedium of the procedure, the preparatory bleeding was done by withdrawing from all the animals a fixed volume of 20 cc./kgm. at the rate of 2 cc./kgm./min., from 2 to 5 hours before beginning the studies reported below. Since fixed volumes were drawn in the preliminary bleeding, with undetermined amounts of circulatory impairment, it cannot be considered a part of the bleeding volume determinations in these studies. All the studies reported here were done on animals who had survived this type of preparatory exsanguination, without replacement, 2 to 5 hours previously. It has not been possible to correlate any of the results with the length of time between preparatory bleeding and the first measurement, within these limits.

Selection of an end-point for the bleeding in the first measurement. The procedure as contemplated in theory requires that some easily recognized level of circulatory impairment be found which will lie a reasonably constant bleeding volume short of the death of the animal. The residual bleeding volume could be predicted at this point on the basis of control studies, without actually withdrawing the residual volume in animals who must be saved for second measurements.

In animals prepared as described above and bled to death at controlled rates between 2 cc./kgm./min. and 2 cc./kgm./4 min., records of mean arterial pressure had the characteristics shown in figure 1. Both the initial and the terminal plateau were observed with all rates, and were also obtained in animals who had not been subjected to preparatory bleeding. The plateaus are the result of progressive restoration of pressure during the intervals between unit withdrawals. Such restoration does not occur during the steeply-sloped middle portion of the record. In this portion of the bleeding, pressure usually continues to fall after a 2 cc./kgm. unit withdrawal is complete. These relationships are more apparent with the slower rates of bleeding.

Table 1 summarizes the data on animals bled to death at the 2 cc./kgm./3 min.

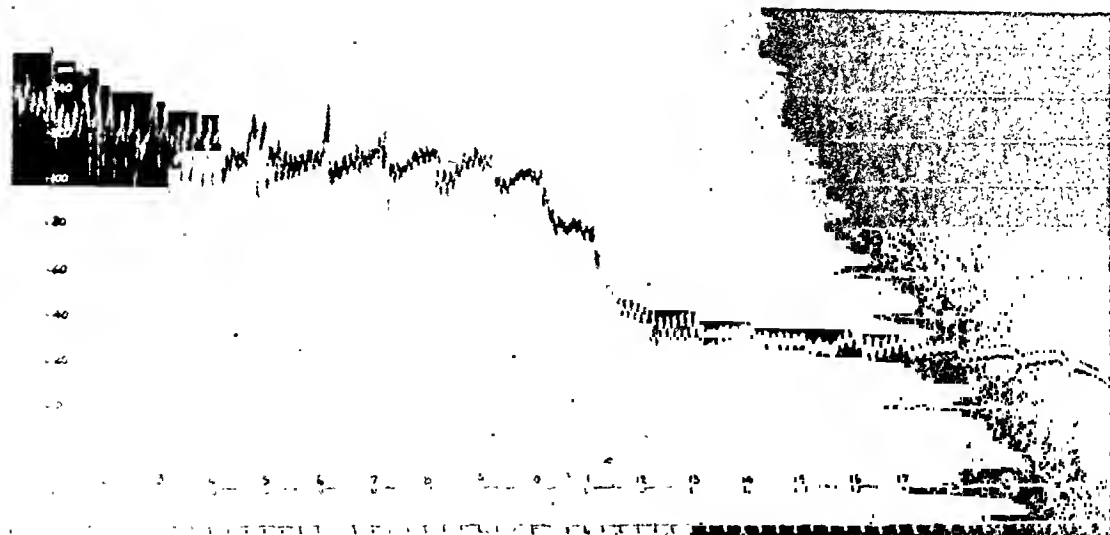


Fig. 1. Effect of hemorrhage at the rate of 2 cc./kgm./3 min. on arterial pressure. Preliminary hemorrhage 20 cc./kgm. at the rate of 2 cc./kgm./min. 4 hours previously. Each numbered signal marks withdrawal of 2 cc./kgm. Time in minutes. Progressive diminution in the amplitude of Mayer waves on the initial plateau is typical.

TABLE 1

Distribution of the volume of blood drawn during fatal hemorrhage with reference to the terminal plateau in arterial pressure (see text and fig. 1)

Hemorrhage rate 2 cc./kgm./3 min. The volume drawn on preliminary hemorrhage, 20 cc./kgm., is not included

DOG NO.	BEGINNING OF TERM. PLAT.	VOL. DRAWN TO BEGINNING OF TERM. PLATEAU	VOL. DRAWN ON TERM. PLATEAU	TOTAL VOL. DRAWN
	mm. Hg	cc./kgm.	cc./kgm.	cc./kgm.
1	26	18	14	32
2	40	20	16	36
3	36	18	16	34
4	42	26	14	40
5	41	22	20	42
6	33	28	16	44
7	47	12	20	32
8	34	20	12	32
9	44	20	20	40
10	47	22	20	42
11	40	16	12	28
12	44	4	16	20
13	36	2	12	14
14	38	6	14	20
15	36	8	16	24
16	45	6	14	20
17	55	8	14	22
Mean.....			15.65	30.71
S.D.....			2.85	9.35
S.E.....			0.69	2.27

rate. If the 20 cc./kgm. drawn from each animal on the preparatory bleeding were added to the volumes recorded in the table, the total volumes obtained and the individual variations would be of the same order of magnitude as those reported for unanesthetized animals bled to death by uncontrolled arterial hemorrhage (Ivy, Greengard, Stein, Grodins and Dutton, 1943). The volume drawn on the terminal plateau is considerably more constant than the volume required to lower pressure to the plateau. The standard deviation of the former value, as shown in the table, is only 18.5 per cent of its mean, while the standard deviation of the total bleeding volume is 30.5 per cent of its mean. It appears from these data that in animals prepared and bled in this way, arrival at the terminal plateau signals production of a circulatory state which can withstand only limited and fairly constant further volume withdrawal. The constancy of length of the terminal plateau cannot be ascribed simply to the fact that all the animals are exposed for the same length of time to damage at these low pressure levels, since animals bled to the expected middle of the plateau and left undisturbed not infrequently lived for 2 hours or longer without either a rise or a fall in pressure. Residual bleeding volume could be predicted with reasonable accuracy on the basis of the data of table 1 if the actual bleeding during the first measurement were terminated anywhere along the lower plateau, at fixed volumes from its beginning.

Although the terminal plateau is easy to identify on most finished records, it is often difficult to demonstrate on tracings which are in progress without invading it for a number of unit bleedings. If the plateau is to be used as an empirical landmark for terminating the first bleeding, it seemed better not to invade it, but to try to obtain a pressure end-point lying just above its beginning on the steeply-sloped portion of the record. Animals were accordingly bled to death at various rates, and an examination made of the constancy of volume withdrawal required to produce death after pressure had been lowered to 60, 50, and 40 mm. Hg. The data for three rates of bleeding are summarized in table 2. Bleeding volume is divided in the table into: H_{1a} , the volume required to lower pressure to the level chosen as trial end-point; and H_{1c} , the volume required to produce death after this level had been passed. Since pressure may continue to fall after a 2 cc./kgm. unit withdrawal is complete, the volumes given in the table are the volumes required to lower pressure below the trial pressure level. The table does not include animals whose pressure was reduced below 60 mm. by withdrawal of less than 12 cc./kgm. They do not appear to be suitable for studies on blood substitutes for reasons which are considered in a subsequent section of this report, and are excluded from all summaries of data except tables 1 and 5. Comparison of the data on the 3-minute rate in the case of those animals which appear in both table 1 and table 2 shows that the 60 mm. end-point usually lies just above the beginning of the plateau, while the 50 mm. end-point is in some cases above, in other cases below this level.

The accuracy with which the first bleeding volume could be obtained by withdrawing blood to any of these pressure end-points and extrapolating for the residual volume, appears from the data of table 2 to decrease as the rate of

bleeding increases. The percentage error would be greatest when minimum values for H_{1a} (actual volume drawn) happened to coincide with maximum deviations of H_{1c} (residual volume) from its mean. If 60 mm. Hg is used as the level for terminating the actual withdrawal (it appears from table 2 to be the most reliable pressure end-point for most of the rates); if minimum H_{1a} volumes are 12 cc./kgm., and if 2 s.d. be taken as the greatest deviation from the mean

TABLE 2

The volume of H_{1a} and H_{1c} obtained at various levels of arterial pressure

All volumes as cc./kgm. Only mean values with standard deviation and standard error are given, except for first group. See text for explanation of symbols.

DOG NO.	RATE REM.	VOLUME H_{1a} WHEN END-POINT IS:			VOLUME H_{1c} , MEASURED FROM END-POINT		
		60 mm. Hg	50 mm. Hg	40 mm. Hg	60 mm. Hg	50 mm. Hg	40 mm. Hg
1	2 cc./kgm./3 min.	14	16	20	18	16	12
2	2 cc./kgm./3 min.	18	22	26	18	14	10
3	2 cc./kgm./3 min.	14	16	20	20	18	14
4	2 cc./kgm./3 min.	26	28	30	14	12	10
5	2 cc./kgm./3 min.	22	22	24	20	20	18
6	2 cc./kgm./3 min.	22	26	28	22	18	16
7	2 cc./kgm./3 min.	14	14	20	18	18	12
8	2 cc./kgm./3 min.	20	20	20	12	12	12
9	2 cc./kgm./3 min.	18	20	24	22	20	16
10	2 cc./kgm./3 min.	20	24	36	22	18	6
11	2 cc./kgm./3 min.	14	16	18	14	12	10
Mean		18.36	20.36	24.18	18.18	16.18	12.36
S.D.		4.08	4.55	5.47	3.51	3.16	3.45
S.E.		1.20	1.37	1.65	1.06	0.95	1.04
NO. DOGS							
7	2 cc./kgm./2 min.	21.71	23.14	25.14	16.57	15.14	13.14
S.D.		8.20	7.90	8.23	4.56	4.73	5.02
S.E.		3.09	2.98	3.10	1.72	1.78	1.89
7	2 cc./kgm./4 min.	18.57	20.86	22.0	10.57	8.29	7.14
S.D.		3.41	3.80	4.61	1.90	3.15	2.54
S.E.		1.29	1.44	1.74	0.72	1.19	0.96

value of H_{1c} , the error will not in any case exceed 17 per cent for the 4-minute rate, 23 per cent for the 3-minute rate, and 32 per cent for the 2-minute rate.

The unavoidable change in bleeding volume following the first measurement. Table 3 summarizes the results obtained when the blood drawn for H_{1a} measurement was reinjected as soon as the end-point was reached. The estimated residual volumes at the end-point for the first bleeding are the H_{1c} values of table 2. The second bleeding volumes (H_2) were measured about 4 hours after completing the replacement of blood, and were obtained by bleeding the animals

completely to death, always at the rate used for the first measurement. The $H_2:H_1$ ratios obtained, where H_1 is taken as the sum of H_{1a} and H_{1c} , have been multiplied by 100 for the table to show the percentage change in bleeding volume under these conditions.

The variations in $H_2:H_1$ appear from the table to become increasingly large as slower rates of bleeding are used for the measurements. Since the accuracy in estimating H_{1c} increases as slower rates are used (see table 2), these variations can hardly be attributable entirely to the error in computing H_1 . For the 2-minute rate, the variations are almost exactly those to be expected from the

TABLE 3

$\frac{H_2}{H_1} \times 100$ when the blood drawn for measurement of H_{1a} is heparinized and reinjected as soon as the measurement is complete

H_1-H_2 interval approximately 4 hours. All volumes in cc./kgm. H_{1c} values obtained from table 2. See text for explanation of symbols.

NO. ANIMALS	RATE HEM.	END-PT. H_{1a}	VOLUME H_{1a}	VOLUME H_{1c}	VOLUME H_2	$\frac{H_2}{H_1} \times 100$
		mm. Hg				
10	2 cc./kgm./2 min.	40	22.4	13.14	31.0	87.4
<i>S.D.</i>			6.10		6.27	7.16
<i>S.E.</i>			1.96		1.98	2.26
8	2 cc./kgm./3 min.	60	17.75	18.18	26.25	73.6
<i>S.D.</i>			3.77		5.20	13.82
<i>S.E.</i>			1.43		1.99	5.23
10	2 cc./kgm./3 min.	60*	23.0	14.18	26.2	69.9
<i>S.D.</i>			4.32		6.35	11.54
<i>S.E.</i>			1.37		2.01	3.65
5	2 cc./kgm./4 min.	60	17.2	10.57	20.0	70.6
<i>S.D.</i>			5.40		9.16	30.08
<i>S.E.</i>			2.41		4.09	13.43

* + 4 cc./kgm.

error in H_1 , but for both the slower rates the scatter in $H_2:H_1$ is much too great to be accounted for in this way. Since the values for $H_2:H_1$ decrease as well as scatter when the slower rates are used, it is tempting to suppose that the slower rates introduce additional variables in the form of some sort of irreversible cardiovascular damage. Neither the mean ratio nor the magnitude of its variations was significantly altered, however, when bleeding at the 3-minute rate was continued until an extra 4 cc./kgm. had been drawn, before replacement (table 3).

The extent of spontaneous recovery when the blood drawn for the first measurement

is not replaced. If changes in bleeding volume are to be used empirically for the evaluation of blood substitutes, it is obviously important to show that low second volumes are obtained when no replacement is made after withdrawal for the first measurement. The experiments of table 3 were accordingly repeated without any replacement after the H_{1a} volumes had been withdrawn. Studies of this sort were not done for the 4-minute rate, since the variations in $H_2:H_1$ obtained with it in the foregoing appeared to be too large for the present purposes. The data are given in table 4. Of the 30 animals listed in the table, only 11 survived the 4-hour interval. Only 3 of these had bleeding volumes at the end of 4 hours which were larger than the residual volumes estimated

TABLE 4

$$\frac{H_2}{H_1} \times 100 \text{ when no replacement is made after withdrawing } H_{1a}$$

H_1 - H_2 interval approximately 4 hours. All volumes in cc./kgm. H_{1a} values obtained from table 2. Symbols as in table 3.

NO. ANIMALS	RATE HEM.	END-PT. H_{1a}	VOLUME H_{1a}	VOLUME H_{1e}	VOLUME H_2	$\frac{H_2}{H_1} \times 100$
		mm. Hg				
10	2 cc./kgm./2 min.	40	20.2	13.14	4.8	15.2
S.D.....			3.19		6.74	21.38
S.E.....			1.01		2.13	6.75
10	2 cc./kgm./3 min.	60	22.8	18.18	7.2	19.6
S.D.....			8.44		8.22	21.36
S.E.....			2.67		2.60	6.72
10	2 cc./kgm./3 min.	60*	25.6	14.18	2.6	6.2
S.D.....			5.32		5.48	13.12
S.E.....			1.37		1.73	4.15

* + 4 cc./kgm.

for them on completing the first bleeding. In none of these did the second volume exceed the value taken for H_{1e} by more than 0.5 s.d.

It is apparent from these data that if a spontaneous rise in bleeding volume above its residual value occurs after withdrawal of H_{1a} , it does not persist for 4 hours. It would appear, therefore, that any value for H_2 in excess of the value for H_{1e} , may under these experimental conditions be attributed to the type of treatment given after withdrawal of H_{1a} .

The behavior of animals with small control bleeding volumes. Six animals appear in table 1 whose arterial pressure was reduced to the terminal plateau on withdrawal of less than 12 cc./kgm. Plateau volumes are somewhat less for them (average 14.33 cc./kgm.) than for the rest of the animals (16.36 cc./kgm.), but are within the limits of variation for the latter. It has become apparent in the course of these studies that animals with very small bleeding volumes

above the plateau constitute a special group. $H_2:H_1$ ratios obtained for them in experiments with reinjected blood and with other fluids are often beyond the limits of variation for the rest of the animals (Lawson and Rehm, loc. cit.). Illustrative data are given in table 5, for replacement with blood. The limits of variation for the other animals in the respective groups are estimated in the table as the mean values found in table 3 plus and minus 2 s.d.

It may be supposed that occasional animals in this group have had arterial pressure lowered to the end-point selected for H_1 measurement by other mechanisms than simple blood withdrawal. Their residual bleeding volumes at the end of H_{1a} withdrawal would probably be excessively large, and they might be expected to yield excessively high $H_2:H_1$ ratios. It may be further supposed that the rest of them are in a state of unusually rapid deterioration. For the present purposes, both types appear to be excluded by confining the studies to animals who require at least 12 cc./kgm. blood withdrawal to lower pressure to 60 mm. on the first bleeding volume determination. After the animals with

TABLE 5

Individual $H_2:H_1$ ratios obtained in autotransfusion experiments on 4 animals whose arterial pressure was lowered to 60 mm. Hg by withdrawal of less than 12 cc./kgm. on H_1 measurement

HEM. RATE	H_{1a} END-POINT	VOL. LOSS AT 60 MM. Hg ON H_1	$\frac{H_2}{H_1} \times 100$	MEAN RATIO FOR GROUP ± 2 S.D.
	mm. Hg	cc./kgm.		
2 cc./2 min.	40	4	0	101.7-73.1
2 cc./3 min.	60	6	34	101.2-46.0
2 cc./3 min.	60*	6	49	93.0-46.8
2 cc./4 min.	60	6	162	130.7-10.5

* ± 4 cc./kgm.

H_{1a} volumes smaller than this have been excluded, the $H_2:H_1$ ratios obtained do not appear to be related to the control bleeding volumes (Lawson and Rehm, loc. cit.). In these studies about one-sixth of the animals have been discarded because of small H_{1a} volumes. This includes animals who died before the first bleeding volume measurement could be made. A total of 4 animals in addition (about 1 per cent) have been excluded because the first bleeding record suggested that the terminal plateau had been invaded by 6 cc./kgm. or more in lowering pressure to 60 mm. In 3 of these animals aberrant low $H_2:H_1$ ratios were obtained. Lesser invasion of the terminal plateau appears from the records to have occurred in a number of instances without significantly affecting the value of $H_2:H_1$.

DISCUSSION. These data show that control measurements of bleeding volume may be made under certain conditions without producing unpredictable persistent changes in the value of a subsequent measurement. The factors which govern resistance to fatal hemorrhage thus appear to be susceptible to laboratory investigation, since the change in resistance under a given set of conditions may be studied in a single animal.

The primary purpose in these studies was not the measurement of absolute values for bleeding volume in normal animals. Such a purpose would have been defeated by the use of anesthesia and by the use of a preparatory fixed-volume bleeding with undetermined amounts of cardiovascular damage. The studies were designed to find conditions under which the change in bleeding volume between the first and the second measurement is a function of the circulating fluid replacement made at the end of the first. The data given here do not prove that these conditions have been found, since they test only the two extremes of replacement, no infusion and infusion of all the blood drawn for the first measurement. Data are given elsewhere (Lawson and Rehm, loc. cit.) which show that the amount of change in bleeding volume between first and second measurement depends upon the type of fluid given in replacement.

Economy of expression in subsequent discussions of bleeding volume may be served by use of the following symbols:

H_1 = Control bleeding volume. It is measured at a stated, controlled rate of bleeding, in animals prepared under standard conditions. It is obtained by drawing blood to a stated level of circulatory impairment (in these studies a level of arterial pressure near the beginning of the terminal plateau) and is equal to the sum of the actual volume drawn in reaching this end-point (H_{1a}) plus the residual volume (H_{1e}). The residual volume is obtained from control animals by continuing the first bleeding past the stated end-point to death. In the studies reported here for the value of H_{1e} , an attempt has been made to obtain a fair sample of the animals to be used, with respect to size, sex, and H_{1a} values. Seasonal and nutritional influences on the value of H_{1e} have not been studied.

R = Fluid given after H_{1a} has been drawn.

H_2 = Second bleeding volume. Unless otherwise stated, it is always measured by drawing blood at the rate used for H_1 measurement, and is continued without interruption to the death of the animal. If it is to be estimated from a partial bleeding volume, as in the case of H_1 , studies will have to be made on the reliability of end-point.

H_1 - H_2 Interval = The interval between arrival at the end-point for H_1 measurement (in these studies R was infused at this time), and the beginning of H_2 measurement.

It is not surprising that bleeding volume declines during the period of observation in these studies with all the rates of bleeding used, so that $H_2:H_1$ is almost always less than 1, even when R is the blood drawn for H_{1a} measurement. The animals had been kept without food and water for 18 to 24 hours, were under barbital anesthesia, and had been subjected to a preparatory hemorrhage of 20 cc./kgm. before beginning the studies. Damage to the cardiovascular system which would result in irretrievable fluid loss or to impairment of vital organs might be expected to occur under these conditions, and to result in progressive decline in bleeding volume even though the partial exsanguination required for H_1 measurement did not in itself do any irreversible damage. That the measured decline is in some way related to the rate of bleeding used in the determinations seems clear from the data. Whether this means that more irreversible damage is done by the slower hemorrhages when they are used for drawing H_{1a} , as it might appear to mean, cannot be decided without further study. It may mean only that different mechanisms control the volumes obtained with slow and

with fast bleeding, and that the rate of deterioration of these mechanisms is not identical.

The 2 cc./kgm./3 min. rate of bleeding has been selected on the basis of these data for use in the studies on blood substitutes. It is a convenient rate to use. The mechanisms which govern the volume obtained with it appear to undergo fairly rapid deterioration under these experimental conditions, since H_2 is only about 70 per cent of H_1 after an H_1 - H_2 interval of 4 hours, when R is the blood drawn for H_{1a} measurement. The rate of deterioration appears to be relatively constant for all animals. Low values for H_2 , with average $H_2:H_1$ ratios approaching 0, are obtained if R is 0.

SUMMARY

Procedures are described which permit bleeding volume, i.e., the volume withdrawn at controlled rates required to produce death, to be measured with usable accuracy without actually killing the animal. The residual volume, which is not drawn on the first measurement, is estimated with increasing accuracy as the rate of bleeding is reduced from 2 cc./kgm./2 min. to 2 cc./kgm./4 min. Second bleeding volumes, measured 4 hours after replacing the blood drawn on the first test, and obtained by withdrawing blood at the same rate until the animal dies, were almost always somewhat lower than the controls under the experimental conditions described. Second bleeding volumes were in the neighborhood of 90 per cent of the controls when the 2 cc./kgm./2 min. rate was used, and in the neighborhood of 70 per cent when the 2 cc./kgm./3 min. rate was used. When the blood drawn for the control measurements was not replaced, second volumes 4 hours later were never significantly larger than the residual volumes estimated at the termination of the control tests, and were usually considerably smaller, with average values approaching 0.

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THE RELATIVE VALUE OF VARIOUS FLUIDS IN REPLACEMENT OF BLOOD LOST BY HEMORRHAGE, WITH SPECIAL REFERENCE TO THE VALUE OF GELATIN SOLUTIONS¹

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Procedures have been described which permit bleeding volume, i.e., the volume of blood withdrawal required at controlled rates to produce death, to be measured twice in the same animal; and conditions have been found which yield relatively low second volumes when the blood drawn for the first test is not replaced (Lawson, 1943). An empirical comparison of blood substitutes may be made by substituting the fluid under investigation for the blood drawn in the first measurement, and comparing the $H_2:H_1$ ratios obtained.

The procedures selected for these studies yield second bleeding volumes somewhat lower than the controls at the end of 4 hours, if the blood drawn during the first test is heparinized and reinjected. The obvious conclusion has been drawn that some mechanism or mechanisms which govern resistance to fatal hemorrhage undergo deterioration under the conditions of the experiments. The $H_2:H_1$ ratios obtained when R (fluid replacement at end of blood withdrawal for first test) is some fluid other than blood cannot be used with assurance to show the influence of the substitute fluid on these same mechanisms. Bleeding volume is an empirical value which is probably influenced by a large number of physiological variables. Thus, if a foreign fluid yields higher $\frac{H_2}{H_1}$ ratios than are obtained when R is the blood drawn, it cannot be concluded that the fluid is actually superior to blood in its direct effect on all the mechanisms which govern bleeding volume. The rate of deterioration of some of these may actually be greater when the substitute is used. Similar difficulties of interpretation are encountered if other empirical values, such as mortality rates, are measured after limited intervals of time. These difficulties are not obviated by measuring conventional values, such as blood volume, cardiac output, or circulatory pressures, since none of them can be regarded as testing an isolated mechanism.

The information obtained by comparing the $\frac{H_2}{H_1}$ ratios appears to provide as legitimate a comparison of blood substitutes as could be obtained by subjecting animals to hemorrhage which would be expected to cause death, injecting the fluid under investigation, and determining the percentage of the group which is still alive at the end of a few hours (Ivy, Greengard, Stein, Grodins and Dutton, 1943; Wiggers, 1943). Since a measured value is obtained for each animal, statistically reliable data are obtained with fewer animals than in

¹ Aided by a grant from the Knox Gelatine Company, Johnstown, N. Y.

survival rate studies which determine only whether an animal is alive or dead at the end of a fixed time interval. Furthermore, if the fluids are to be given to subjects who lose circulating fluid after the injection those fluids which yield the larger bleeding volumes in these tests might reasonably be expected to support life longer than those which yield small bleeding volumes. This would not necessarily be true if, under conditions of clinical use, types of circulatory damage are encountered which are aggravated by the fluids, and which are not produced in the animals used for the tests. In these studies any simulation of clinical states in which blood substitutes might be employed is incidental.

METHODS. The procedure already described in detail (Lawson, loc. cit.) was carried out on barbitalized dogs without modification except that the fluid under investigation was substituted for autotransfused blood in the replacement (R) following the first bleeding volume measurement. The volume of R was always equal to the volume of blood actually drawn during the first measurement (H_{1a}). Blood withdrawal for both H_1 and H_2 determination was done at the rate of 2 cc./kgm./3 min. On H_1 determination actual blood withdrawal was discontinued as soon as arterial pressure fell below 60 mm. Hg. The residual bleeding volume at this time (H_{1c}) was taken from the previously reported control studies as 18.18 cc./kgm., and H_1 was computed as the sum of H_{1a} and H_{1c} . The H_1 - H_2 interval was approximately 4 hours. Measurement of H_2 was made by bleeding the animal actually to death.

The blood, plasma and serum used in these studies were obtained by bleeding donor dogs to death under barbital anesthesia, pooling the freshly drawn blood from 5 to 8 donors before further processing. These fluids were chilled as soon as possible (serum and defibrinated blood as soon as clotting was complete) and refrigerated until used. They were used within 8 hours after obtaining the blood from the donors.

The two gelatins selected for study are oppositely charged colloids at the pH of blood.² Gelatin VII-54 is prepared from pork skin, and has an isoelectric point in the neighborhood of pH 8. A physically similar pork skin gelatin was studied by Ivy et al. (loc. cit.) under the designation U-17578. Gelatin B-78-1 is prepared from beef bone, and has an isoelectric point near pH 5. Its use as a blood substitute has been studied by Parkins and Lockwood, 1943; Little and Wells, 1943, and Little and Dameron, 1943. A similar gelatin was used in the preliminary studies of Gordon, Hoge and Lawson, 1942. The gelatins were dissolved in 0.9 per cent NaCl to a concentration of 3.45 per cent for B-78-1 and a concentration of 4.85 per cent for VII-54, and pH was adjusted to 7.0-7.3 by addition of N/10 NaOH. At these concentrations the solutions had a colloidal osmotic pressure of 22.5 mm. Hg, which was approximately the same as that of the hematogenous fluids used in the study. Colloidal osmotic pressures were measured at 37°C. by a modification of the method described by Hepp (1936), using tight membranes with an average permeability equal to 11.2×10^{-3} in the units recommended by Wells (1932).

RESULTS. The data obtained are summarized in table 1, which also includes,

² All supplies of gelatin were furnished by the Knox Gelatine Company, Johnstown, N. Y.

TABLE 1

$$\frac{H_2}{H_1} \times 100 \text{ with different types of replacement fluid}$$

H_1 - H_2 interval 4 hours. Values given are the means, with their standard deviations (S.D.) and standard errors (S.E.). See text for explanation of symbols in headings.

REPLACEMENT FLUID (R) VOL. = VOL. H_{1a}	NUMBER OF ANIMALS	H_{1a}	H_{1c}	H_2	$\frac{H_2}{H_1} \times 100$
		cc./kgm.	cc./kgm.	cc./kgm.	
0	10	22.8	18.18	7.2	19.6
S.D.		8.44		8.22	21.36
S.E.		2.67		2.60	6.72
Autotransfused blood	8	17.75	18.18	26.25	73.6
S.D.		3.77		5.26	13.82
S.E.		1.43		1.99	5.23
0.9% NaCl.	10	21.6	18.18	14.8	37.6
S.D.		4.81		7.73	18.07
S.E.		1.52		2.44	5.69
Heparinized blood	10	20.6	18.18	27.2	70.7
S.D.		4.21		5.09	13.57
S.E.		1.33		1.58	4.30
Defibrinated blood	8	21.0	18.18	24.8	62.8
S.D.		5.23		7.24	14.66
S.E.		1.85		2.56	5.18
Heparinized plasma	10	19.4	18.18	25.8	68.1
S.D.		4.52		5.99	9.87
S.E.		1.43		1.90	3.12
Serum.	9	16.4	18.18	24.4	70.5
S.D.		7.06		6.98	16.28
S.E.		2.49		2.47	5.75
Gelatin VII-54, 4.85%	8	23.0	18.18	25.3	60.5
S.D.		8.48		9.55	16.03
S.E.		3.00		3.38	5.67
Gelatin B-78-1, 3.45%	7	16.3	18.18	21.1	61.1
S.D.		3.14		3.80	7.84
S.E.		1.18		1.43	2.96

for comparison, previously reported data obtained when R is the blood drawn for H_{1a} measurement, and when R is 0 (no infusion).

The probability that random sampling variations account for the differences in $\frac{H_2}{H_1}$ obtained when R is 0.9 per cent NaCl and when R is 0 is approximately 0.06. The probability that the observed differences between 0.9 per cent NaCl and the remaining fluids are due to variations of this sort is in every case less than 0.02 (Fisher, 1941). Attempts were made to obtain fair samples by avoiding any selection of animals within the limits previously described, the animals being used in whatever studies were in progress as they were delivered to the laboratory. The possibility of seasonal variations in the value of H_{1a} has been suggested by more recent observations, and has not been fully explored. All the comparative data reported here were obtained within a single season, and determination of H_{1a} was done concurrently.

No statistically significant differences are observed in the table among the various colloidal solutions employed. The suggestively low values obtained with defibrinated blood require further study in view of the possibility that partially hemolyzed blood may be inferior as an infusion fluid (DeGowin, 1942).

Temporary depressor effects were usually obtained with pooled blood, plasma, and serum, but not with autotransfused blood. Neither the data of the table, nor examination of individual responses reveals any correlation between these effects and the $\frac{H_2}{H_1}$ ratios obtained.

Although the two gelatin solutions yield about the same mean value, much lower values for $\frac{H_2}{H_1}$ were obtained in some of the animals receiving VII-54 than were ever observed with B-78-1 (see s.d. in table). Whether this means that VII-54 has positive harmful effects in occasional animals, or simply that it fails to be retained as circulating colloid, cannot be decided without further study. The differences shown in the table between the gelatin solutions on the one hand and heparinized blood, plasma, and serum, on the other, are in general agreement with comparative data which have been obtained on one of them (VII-54) in the treatment of animals subjected to single massive hemorrhage, using 24-hour survival rates as an index to adequacy of blood substitution (Ivy et al., loc. cit.).

The relationship between $\frac{H_2}{H_1}$ and the volume of H_{1a} . It has already been shown that the magnitude of the ratio (i.e., the rate of decline in bleeding volume during the 4-hour H_1 - H_2 interval) is not related to the control bleeding volume, if the latter is such that H_{1a} is 12 cc./kgm. or greater, in experiments in which R is the animal's own blood (Lawson, loc. cit.). It cannot be assumed, however, that this will also be true when R is a foreign fluid. The volume of fluid which each animal received is always, in the studies reported here, equal to the volume of H_{1a} . If the fluids given are toxic in the customary sense, the animals with the larger H_{1a} volumes and who receive correspondingly larger volumes of the fluids might be expected to yield the lower $\frac{H_2}{H_1}$ ratios. These relationships are examined

in figure 1 for the two gelatin solutions and for defibrinated blood, which might be regarded as most likely, of all the fluids studied, to possess some sort of harmful effect. The figure fails to show any relationship between the volume of H_{1a} , and therefore the volume of R, and $\frac{H_2}{H_1}$. Similar data have been obtained for the other fluids studied.

These data are not presented to show that harmful effects are absent, but simply that within the very limited dosage range covered by variations in the volume of R when the latter is made equal always to H_{1a} , the effects do not appear to influence the value of $\frac{H_2}{H_1}$. Comparisons such as those given in table 1

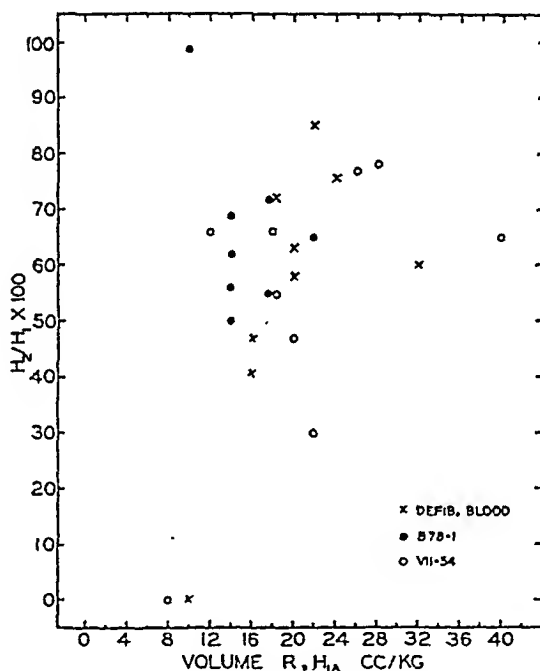


Fig. 1. Relationship between $\frac{H_2}{H_1}$ and the volume of R when R is equal to H_{1a} , in experiments on defibrinated blood (pooled), gelatin VII-54, 4.85 per cent in 0.9 per cent NaCl, and gelatin B-78-1, 3.45 per cent in 0.9 per cent NaCl.

are therefore valid, for all the fluids studied, even though identity of H_{1a} is not obtained with these small groups of animals.

The 3 animals shown in figure 1 whose H_{1a} volumes were less than 12 cc./kgm. demonstrate the wide variations in $\frac{H_2}{H_1}$ obtained when such animals are used, which were discussed in the previous report. Animals of this sort are not included in any other summaries of data in this report.

The value of various grades of gelatin. A series of increasingly degraded gelatins was prepared from a single high average mol. wt. gelatin, by exposing stock solutions of the latter to temperatures of 115.5°C. (10 lbs. autoclave pressure) for increasing lengths of time up to 3 hours. The stock solutions

were made from gelatin B-78-1, or from a duplicate manufacturer's batch. pH was adjusted to 7.4 before autoclaving, the pH range of the solutions after autoclaving being 6.9 to 7.3.

The data on this series are summarized in figure 2, in which the gelatin concentration of the prepared solutions was adjusted to 3.45 grams/100 cc. before use. It is apparent from the figure that complex changes occur in the value

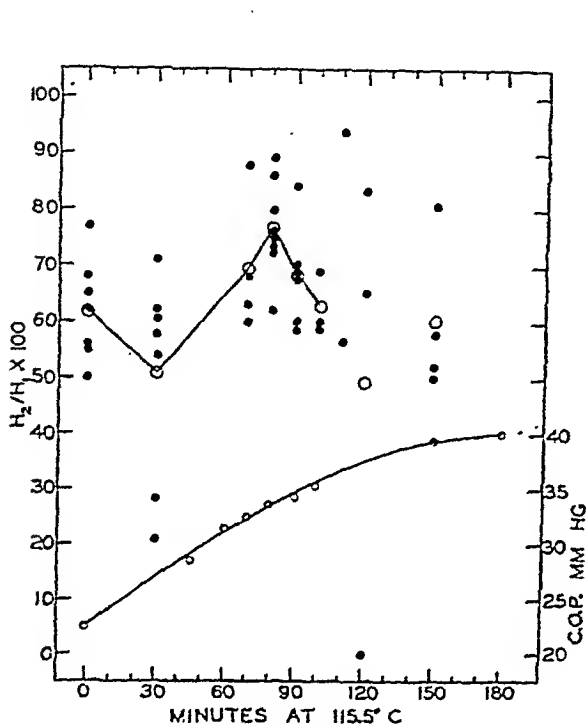


Fig. 2

Fig. 2. The effect of heat treatment (115.5°C.) on the value of 3.45 per cent solutions of gelatin B-78-1 in 0.9 per cent NaCl, as shown from the values obtained for $\frac{H_2}{H_1}$ when R is the

heat treated solution. Mean values for the animals receiving a solution are given by the large hollow circles, and are connected to facilitate reading the figure. Small hollow circles on the lower curve give colloidal osmotic pressure (at right) in millimeters of mercury.

Fig. 3. Same as figure 2 except that the concentration of all solutions was adjusted to give colloidal osmotic pressure = 33.5 mm. Hg. The gelatin concentration of the solutions, in grams/100 cc., is given at the top of the figure. The group of animals receiving the 80-minute solution is the same as in figure 2.

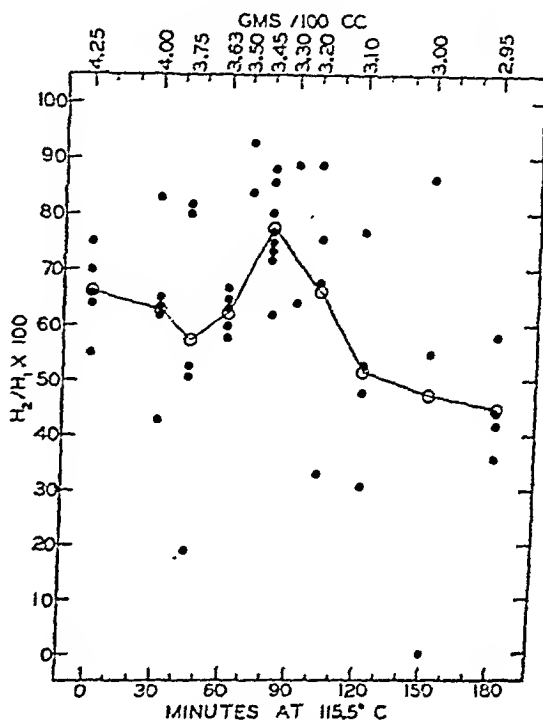


Fig. 3

of gelatin, as measured by its effect on bleeding volume, when the high molecular weight gelatin is subjected to degradation.

The possibility that some of these changes in value are due to the fact that colloidal osmotic pressure increases progressively in the series (see fig. 2) was investigated by repeating the study with solutions whose gelatin concentration had been adjusted to iso-osmolarity; 33.5 mm. Hg was chosen as the level of colloidal osmotic pressure to be used for this purpose, since the scatter in the data appeared to be least at this level (fig. 2). Data on the iso-osmolar series are summarized in figure 3, which also shows the concentration, in grams/100 cc.,

of each solution. None of the characteristics of the first series appears to have been significantly altered by adjustment of concentration to this level of colloidal osmotic pressure.

DISCUSSION. The data given here supplement those given in the previous report by showing that the change in bleeding volume under these experimental conditions is a function of the nature of the replacement fluid R. The second bleeding volume is evidently not simply a measure of the volume of water which has been given to the animal after the first measurement. The significant differences in $\frac{H_2}{H_1}$ when R is a colloidal solution and when it is crystalloidal

(NaCl) warrant the tentative conclusion that colloid is a limiting factor for bleeding volume in animals bled to these levels and given replacement fluids.

Comparison of the data obtained with cell-free and with cellular fluids prepared from blood suggests that the blood cells are not a limiting factor in animals who have lost blood in these volumes (20 cc./kgm. on preparatory bleeding, plus the volume of H_{1a}). Studies which are now in progress suggest that they may become a factor if the end-point for the first measurement is lowered.

That the type of colloid used is important for the second bleeding volume is shown by comparing the data on the hematogenous fluids with the data on gelatin, and by examination of the values obtained with different grades of gelatin in iso-osmolar solution. The low values obtained with the more fully degraded gelatins may be due to their diffusibility. The reason for the other changes in value in the degraded series is obscure.

These studies fail to reveal any difference in the effectiveness of plasma and serum such as has been reported when they are used to maintain life for 24 hours in animals subjected to single rapid hemorrhage (Ivy et al., loc. cit.). On the basis of the information available, it is not possible to decide whether real differences exist which do not reveal themselves within the 4-hour period of our studies, or whether the reported differences were due to methods of preparation of the fluids.

SUMMARY

The change in bleeding volume under conditions which have been described previously has been used for comparing certain fluids as blood substitutes.

Comparisons were made on the basis of the $\frac{H_2}{H_1}$ ratios obtained when these fluids were used to replace the blood drawn on the first bleeding volume determination. The mean percentage ratios obtained in groups of 7-10 animals, with their standard errors, were: No fluid, 19.6 ± 6.72 ; autotransfused blood, 73.6 ± 5.23 ; pooled heparinized blood, 70.7 ± 4.30 ; pooled heparinized plasma, 68.1 ± 3.12 ; pooled defibrinated blood, 62.8 ± 5.18 ; pooled serum, 70.5 ± 5.75 ; 0.9 per cent NaCl, 37.6 ± 5.69 ; 4.85 per cent pork skin gelatin in 0.9 per cent NaCl, 60.5 ± 5.67 ; 3.45 per cent beef-bone gelatin in 0.9 per cent NaCl, 61.1 ± 2.96 .

The value of the bone gelatin undergoes complex changes with heat-degrada-

tion at a temperature of 115.5°C ., which are observed both when fixed concentrations are used and when the solutions are adjusted to iso-osmolarity. Peak values were obtained with the partially degraded materials.

It is concluded that bleeding volume under these experimental conditions is a function of the amount and type of colloid in the replacement fluid, and that the cellular content of the replacement fluid is not a limiting factor.

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THE EFFECT OF THE SODIUM CHLORIDE INTAKE ON THE WORK PERFORMANCE OF MAN DURING EXPOSURE TO DRY HEAT AND EXPERIMENTAL HEAT EXHAUSTION¹

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In contrast to the extensive work on the rôle of sodium chloride in the pathogenesis and treatment of heat cramps (1), the relationship of the NaCl intake to heat exhaustion and cardiovascular function of men working in heat has received only incidental attention (2, 3). This communication will deal with the effects of work in the heat on cardiovascular and related functions and the incidence of heat exhaustion. Thirty-four normal young men lived and worked at high temperatures under rigidly controlled conditions on three different levels of NaCl intake. Reference will also be made to 15 additional subjects who were studied during 92 subject days. The variables measured were pulse rates in rest and work, rectal temperature, rate of sweating, cardiovascular postural adjustment tests, the concentration of chloride in sweat, plasma and urine, blood urea nitrogen and in some cases the refractive index increment of the plasma.

METHODS AND MATERIALS. Details of the regimen under which the subjects ate, slept and worked in a controlled temperature suite have been given in a previous report (4). In brief, control observations were obtained when the dry bulb temperature was 80°F. and the wet bulb 65°F. Hot conditions were represented by a dry bulb temperature of 120°F. and wet bulb 85°F. Night conditions, 6 p.m. to 8 a.m., during the hot period were 85° to 95°F. dry bulb, 65° to 75°F. wet bulb. Approximately an hour and a half was required for a complete change in temperature. In all experiments, work consisted of walking on a motor driven treadmill at 3.25 miles per hour at 7.5 per cent grade for 6 ten-minute periods alternating with 10 minute rest periods for each half day. "Work" pulse rates were obtained by counting the pulse for the first 15 seconds after each work period. Rectal temperatures were taken each half day at the end of the third and sixth work periods. Rate of sweating was measured over the third period in the morning and the sixth period in the afternoon. Subjects were weighed nude and dry to ± 7 grams. Modified Crampton (4, 5) blood ptosis tests were carried out before breakfast and 20 minutes after the end of work in the afternoon. In all experiments records were kept of water and fluids

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consumed and 24-hour urine samples were collected each day. Body weights, with an empty bladder, were obtained and venous blood was drawn each morning before breakfast. Electrocardiograms were taken on subjects who were on the low NaCl intake during the control period, at the end of the experiment and on all subjects who had marked heat exhaustion. Arm-plus-hand sweat was collected in shoulder-length "neoprene" gauntlets during the second and third work periods each morning and afternoon.

Three series of experiments were carried out. The subjects in series I were 22 soldiers of the 710th M.P. Battalion. Ten of these men were studied on a low NaCl intake (5.8 ± 2 grams) and 12 were studied on a moderate (14.7 ± 2 grams) intake. The duration of the hot period was $3\frac{1}{2}$ days preceded by a control period of 24 hours. In series II, six hired male students of the University were studied during 2 hot periods of 2 days each 3 weeks apart. In each period half of the men were on the low and half on the moderate NaCl regimen, the order being reversed in the second experiment. In this way each subject served as his own control. Series III was similar except 6 University subjects alternated on the high (30 ± 2 grams) and the moderate NaCl intakes. Each hot period in series II and III was preceded by a 2-day control period.

All subjects received a standard diet which contained, by daily analysis, 5.8 ± 2 grams of NaCl. This diet supplied 3100 calories and was adequate, according to ordinary standards, in protein and vitamins. The moderate and high NaCl intakes were achieved by giving in addition 8 and 24 grams of NaCl respectively with the meals. Water was allowed ad lib. except during actual observations and work on the treadmill.

Chlorides were estimated by the mercurimetric titration of Cavett and Holdridge (6) and the method of Keys (7). Food and urine were ashed before analysis. Sweat was analyzed directly. The concentration of plasma chlorides was determined on plasma separated from oxygenated blood. Urea analyses were carried out by a modified Folin-Svedburg method. Total solids (8) of the plasma were estimated with a Pulfrich dipping refractometer in a constant temperature water bath at 30°C .

The statistical significance of differences between means of physiological data was evaluated by means of the t-value (9).

Results in work. The average work pulse rates and rectal temperatures for series I are given in table 1. Statistically significant differences between the two groups for work pulse rates demonstrated a definite advantage for the subjects on the moderate NaCl intake. The rectal temperature differences, although not statistically significant, favor the moderate NaCl group. Results of series II are given in table 2A. The average results show the same advantage for subjects on the moderate NaCl intake.

The results of series III are given in table 2B. No significant differences were found between the work pulse or rectal temperature of six subjects consuming 15 grams of NaCl and the same men consuming 30 grams of NaCl a day.

Cardiovascular results in rest. The average values for pulse rates and blood pressures before and after elevation on the tilt table morning and evening are

given in table 3 along with the average Crampton scores. This table does not include those men who failed to complete the day's work or who fainted before observations could be completed. Differences in the pulse rates of the two groups (subject tilted) appeared on the afternoon of the first day in the heat. This difference became significant on the second afternoon and was still apparent on the third afternoon. The only significant difference in the blood pressures appeared on the afternoon of the second day in the heat (subject tilted).

The moderate NaCl group showed an advantage over the low NaCl group by making a statistically significant improvement in Crampton score between the afternoon of the first day and the afternoon of the second day of the hot period; while, in contrast, the low NaCl group failed to show any improvement. The

TABLE 1

*The average work pulse rates and rectal temperatures for 10 men on the low sodium chloride (6 ± 2 grams) intake and for 12 men on the moderate sodium chloride (15 ± 2 grams) with the *t* values of the differences between groups*

The 1 per cent level of significance is 2.84

	CONTROL	DAYS OF HOT PERIOD						
		1		2		3		4
		a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.
Work pulse rates								
Low NaCl....	131	158	174	159	165	157	158	154
Moderate NaCl	133	148	157	144	148	139	143	130
Δ	2	-10	-17	-15	-17	-18	-15	-24
<i>t</i> value		1.69	2.99	3.04	4.00	3.64	3.80	4.55
Rectal temperature								
Low NaCl....	100.6	100.5	101.2	101.1	101.5	101.3	101.6	100.9
Moderate NaCl.....	100.3	100.7	101.2	100.7	101.1	100.7	101.1	100.3
Δ	-0.3	+0.2	0	-0.4	-0.4	-0.6	-0.5	-0.6
<i>t</i> value.....					0.398	0.659	0.413	

significance of differences between the means is given in terms of the *t*-value in table 4.

Water balance. The water consumption, body weights, urine volumes, total 24-hour sweat volumes and rate of sweating during work are presented in table 5 for the two groups in series I. The men on the low NaCl intake lost more than twice as much weight, drank less water and sweated less than those on the moderate chloride intake. The two groups produced sweat at approximately the same rate on the first day. However, the low NaCl group failed to drink an adequate amount of water and ended the first 24 hours with a dehydration of 2.5 per cent of the body weight as compared to only 0.77 per cent body weight loss for the moderate chloride group. This was associated with a marked net

loss of NaCl (-8.39 grams) in the low NaCl group (see below) and only a slight net loss (0.53 grams) for the moderate group. The failure of the thirst mechanism in the low NaCl group to demand adequate water is in agreement with other observations (10, 11).

On the second and third days the rate of sweating and the 24-hour sweat production fell off markedly in the low NaCl group and showed no significant change

TABLE 2A

Average pulse rates and rectal temperatures for 6 men in series II studied in two periods of two days each in the heat while on low and moderate NaCl intakes

	CONTROL	DAYS OF HOT PERIOD			
		1		2	
		a.m.	p.m.	a.m.	p.m.
Work pulse rates					
Low NaCl (6 ± 2 grams).....	114	140	151	143	154
Moderate NaCl (15 ± 2 grams).....	119	140	148	132	141
Δ	± 5	0	-3	-11	-13
Rectal temperatures					
Low NaCl (6 ± 2 grams).....	100.1	100.3	100.7	100.8	101.2
Moderate NaCl (15 ± 2 grams).....	100.0	100.2	100.6	100.1	100.6
Δ	-0.1	-0.1	-0.1	-0.7	-0.6

TABLE 2B

Average pulse rates and rectal temperatures for 6 men in series III studied in two periods of two days each in the heat while on moderate and high NaCl intakes

	CONTROL	DAYS OF HOT PERIOD			
		1		2	
		a.m.	p.m.	a.m.	p.m.
Work pulse rate					
Moderate NaCl (15 ± 2 grams).....	107	136	148	134	143
High NaCl (30 ± 2 grams).....	103	136	147	132	139
Δ	-4	0	-1	-2	-4
Rectal temperature					
Moderate NaCl (15 ± 2 grams).....	100.1	100.6	101.2	100.5	100.9
High NaCl (30 ± 2 grams).....	99.8	100.3	101.2	100.3	100.7
Δ	-0.3	-0.3	0.0	-0.2	-0.2

in the moderate NaCl group. The reduction in the total sweat volume of the low NaCl group is accounted for by reduced sweat loss during work.

NaCl metabolism. Table 6 gives the estimated NaCl balances of the two groups in series I. The following assumptions were made in preparing this table: 1, the difference between the NaCl intake and NaCl loss in the urine appeared in the sweat; 2, the changes in body weight represented a loss or gain of a

TABLE 3

The average systolic blood pressures and pulse rates before and after elevation on a tilt table and the average modified Crampton scores of 10 men on the low (6 ± 2 grams) NaCl regimen compared with 12 men on the moderate (15 ± 2 grams) NaCl regimen before and during a $3\frac{1}{2}$ day exposure to dry heat

A.M. values were obtained before breakfast and p.m. values 20 minutes after the last work period in the afternoon

DAY	TIME	DIET	NO. OF MEN	BLOOD PRESSURE		PULSE RATE		CRAMPTON SCORE
				Before	After	Before	After	
Control	p.m.	Low	10	124	115	73	96	56
		Moderate	12	121	112	77	96	60
				—	—	—	—	—
	a.m.	Low	10	120	118	63	87	63
		Moderate	12	122	117	77	83	66
				—	—	—	—	—
I (hot)	p.m.	Low	7	117	99	98	128	41
		Moderate	12	113	100	94	118	46
				—	—	—	—	—
	a.m.	Low	10	119	109	68	93	53
		Moderate	12	121	111	73	91	61
				—	—	—	—	—
II	p.m.	Low	9	115	102	96	131	44
		Moderate	11	120	113	90	108	61
				—	—	—	—	—
	a.m.	Low	10	123	112	66	96	45
		Moderate	11	118	109	70	90	62
				—	—	—	—	—
III	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
IV	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
V	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
VI	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
VII	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
VIII	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
IX	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
X	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XI	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XII	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XIII	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XIV	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XV	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XVI	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XVII	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XVIII	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XIX	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XX	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XXI	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XXII	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XXIII	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XXIV	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XXV	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XXVI	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XXVII	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XXVIII	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XXIX	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XXX	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XXXI	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XXXII	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XXXIII	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XXXIV	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XXXV	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
XXXVI	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66

TABLE 4

Analysis of the results of the postural adjustment tests in terms of the t-value

VARIABLE	TIMES OR CONDITIONS COMPARED	DEGREES OF FREEDOM	t VALUE CALCULATED	t VALUE AT 5 PER CENT LEVEL OF SIGNIFICANCE
Blood pressure subject tilted	2nd p.m. hot period "Low" vs. "Mod." chloride	18	2.27	2.10
Pulse rate subject tilted	2nd p.m. hot period "Low" vs. "Mod." chloride	18	5.34	2.10
Crampton score	"Low" chloride group 1st p.m. vs. 2nd p.m. of hot period	8	0.33	2.31
Crampton score	"Mod." chloride group 1st p.m. vs. 2nd p.m. of hot period	10	3.77	2.26

TABLE 5

Average morning body weights, water consumption, urine volume, total 24 hour sweat volume and rate of sweating during work of the low chloride group and the moderate chloride group (series I)

VARIABLE	NaCl INTAKE	CONTROL	DAYS OF EXPOSURE TO HEAT		
			1	2	3
Average body weight*.....	"Low"	157.4	153.4	152.2	151.8
Per cent Δ from control.....			2.5	3.3	3.6
Average body weight*.....	"Mod."	154.0	152.8	151.7	152.6
Per cent Δ from control.....			0.77	1.5	1.3
Water intake (liters).....	"Low"		4.82	5.51	5.61
	"Mod."		5.73	6.27	6.33
Urine volume (liters).....	"Low"		0.486	0.687	0.670
	"Mod."		0.656	0.648	0.491
Total sweat volume (liters)†.....	"Low"		6.44	6.12	5.87
	"Mod."		6.37	6.87	6.27
Rate of sweating in work (gms. per minute).....	"Low"	a.m.	14.8	12.0	11.1
		p.m.	11.6	10.7	10.4
	"Mod."	a.m.	14.4	14.7	15.8
		p.m.	14.7	15.0	15.0

* Values for body weight represent values with an empty bladder before breakfast after completion of specified number of days' exposure to heat. Body weights expressed in pounds.

† Sweat loss equals water drunk plus water in food plus water metabolism minus the sum of urine water plus fecal water plus water lost through the lungs, corrected for 24 hour weight change.

weight losses are from the extra-cellular fluid phase results in maximal figures for the estimated 24-hour NaCl loss in sweat and from all sources.

The estimated 3-day NaCl deficit for the low NaCl group was 12.71 grams as compared to only 2.84 grams for the moderate NaCl group. The evidence gained from series III appears to indicate that NaCl deficits of 2 to 3 grams have no appreciable effect on the ability of men to perform work in the heat. The low chloride group showed a marked conservation of NaCl when compared to the moderate group, losing only 29.65 grams of salt in three days as compared to 44.78 grams for the men on the higher NaCl intake.

Results in blood and plasma. Table 7 gives the findings of the measured blood constituents in the two groups in series I. The total plasma solids as measured by the refractive index increased 15.2 per cent on the morning of the second day

TABLE 6

The estimated NaCl balances of the low and moderate NaCl groups in series I (see text)

All figures are average grams of NaCl per 24 hours, except weight change which is in kilograms. The numbers in the vertical column heads refer to days of hot period.

	LOW NaCl GROUP			MODERATE NaCl GROUP		
	1	2	3	1	2	3
NaCl intake.....	5.30	5.84	5.80	15.45	13.19	13.20
Urine NaCl.....	3.60	1.20	0.45	5.20	2.95	2.45
Weight change (kgm.).....	-1.36	-0.54	-0.18	-0.54	-0.54	+0.14
Equivalent NaCl loss*.....	8.39	3.36	1.04	3.63	3.15	+0.84
Sweat NaCl loss†.....	10.09	8.27	6.04	13.88	13.39	9.91
Total NaCl loss‡.....	13.69	9.47	6.49	16.08	16.34	12.36
NaCl balance.....	-8.39	-3.63	-0.69	-0.53	-3.15	+0.84

* Calculated on the assumption that a gain or loss of weight is due to a gain or loss of extra-cellular fluid. The concentration of chloride in the extra-cellular fluid phase was calculated from plasma chloride concentration.

† Sweat NaCl loss = NaCl intake - urine output \pm the NaCl gain or loss equivalent to the weight change.

‡ Total NaCl loss = sweat NaCl loss + urine NaCl loss.

in the low NaCl group but there was later a tendency to return to normal in spite of progressively increasing dehydration. The slight decrease in the hemoglobin suggests blood destruction in the low NaCl group. The increase in blood urea nitrogen in the low NaCl group was accompanied by a 12 per cent increase in daily urinary urea excretion; in contrast the moderate NaCl group showed a 35 per cent reduction in urea eliminated in the urine. The significance of these findings is not clear.

Sweat chlorides. Lehmann (12) has reported a linear relationship between rectal temperature and the concentration of chloride in the sweat. The present experiments were not strictly comparable but they offer small support to this thesis. The average results on 12 men on the moderate NaCl intake (series I) show at best only a rough correlation: $R = 0.655$. The low NaCl group (series I) showed an inverse relationship: $R = -0.790$.

In the experiments reported here there was a marked decrease in the concentration of chloride in the sweat in all groups during the first days of exposure. The average concentration of chloride in the sweat on the morning of the fourth day in the heat was 55 per cent and 38 per cent of the average concentration of chloride in the sweat on the morning of the first day in the moderate and low NaCl groups (of series I) respectively.

Heat exhaustion. Seven of the 49 men who took part in these experiments suffered heat exhaustion of various grades. The symptoms and objective findings are presented in table 8.

Three other cases are included from groups under substantially the same conditions but not reported here in detail. In all there were 39 men on the moderate NaCl regimen and of these only one suffered definite heat exhaustion and

TABLE 7

The concentration of hemoglobin and urea nitrogen in the blood, chlorides in plasma and the refractive index increment of plasma of the low and moderate chloride groups in series I

DETERMINATION	DIET GROUP	CONTROL	DAYS* OF EXPOSURE TO HEAT		
			1	2	3
Refractive index increment†	Low chloride	0.0171	0.0197	0.0187	0.0183
	Moderate chloride	0.0173			0.0175
Hemoglobin (grams per 100 cc.)	Low chloride	16.02	15.7	15.8	15.7
	Moderate chloride	15.4			15.7
Plasma chlorides (mgm. NaCl 100 cc.)	Low chloride	617	613.2	585	582
	Moderate chloride	637			635
Blood urea nitrogen (mgm. per 100 cc.)	Low chloride	12.0	19.8	20.6	20.7
	Moderate chloride	13.8			14.7

* Values represent findings in blood drawn in morning before breakfast after completion of specified number of days' exposure to heat.

† Refractive index of plasma minus the refractive index of water.

prostration while one other man had to interrupt the work schedule because of mild heat exhaustion. In contrast, 5 men of the 20 on the low NaCl regimen suffered heat prostration and 3 others suffered heat exhaustion requiring interruption of the work schedule. The one man (Du) who developed heat prostration in the moderate NaCl group was 46 years old and the oldest man studied. Du had a very high rate of sweat production. Du lost 23.4 grams of sweat per minute in work as compared to an average of 14.9 grams/min. for all men in the moderate NaCl group. He showed a net loss of 5.5 per cent of his body weight on the first day in the heat and as a result he was badly dehydrated when he collapsed during the morning of the second day.

Case Wa is interesting because he represents heat exhaustion which occurred during a simultaneous increase in the rate of sweating. The routine rate of

TABLE 8

The symptoms, pulse rate, rectal temperature, blood pressure, blood hemoglobin and plasma chlorides of 10 men who suffered various grades of heat exhaustion during a 3½ day exposure to heat

All subjects except Du were on the low chloride regimen

SUBJECT	DAY OF HOT PERIOD	SYMPTOMS	WORK PULSE RATE	WORK RECTAL TEMP.	REST-ING PULSE RATE	REST-ING PRES-SURE		BLOOD HEMO-GLOBIN	PLASMA CHLORIDES
								<i>grams per cent</i>	<i>mgm. per cent</i>
Dar	p.m. 1st	Nausea, vomiting, vertigo, headache	204	102	120	116/70			584
Cel	p.m. 2nd	Nausea, vomiting, vertigo, very slight cramps	156	101			a.m. ill	16.9 18.2	593 567
He	p.m. 1st	Refused to continue, complained of dyspnea	180	102.8					
Ma	p.m. 3rd	Nausea, vomiting, vertigo, weakness	168	100.2	88	96/70	a.m. ill	15.9 17.0	570 618
Wa	a.m. 1st	Fainted before going on treadmill. Fourth period rate of sweating 32/grams/min.*	140	99.2			a.m. ill	17.0 16.55	629 620
Tw	p.m. 2nd	Refused to continue, slightly hysterical	172	101.6			a.m. ill	14.5 14.5	615 609
Gol†	a.m. 3rd	Vomiting, nausea			104	108/90			
Be†	p.m. 3rd	Vomiting, nausea, vertigo, jaundice							
Du‡	a.m. 2nd	Vomiting, vertigo, nausea, marked weakness	160	101.6	116		a.m. ill	15.6 20.07	640 610
Ne§	p.m. 1st	Weak, refused to continue	174	103.6	132	122/60			

* Five subsequent determinations of rate of sweating averaged 12.8 gms./min.

† These men took part in preliminary low chloride experiments and are not included in series I, II, or III.

‡ Forty-six years old; on the moderate chloride diet.

§ Took part in an 8 day experiment previously reported in detail Taylor, Henschel and Keys, Report to N.R.C. April, 1943. This Journal, 1943. (In press.)

sweating was measured over the third period of work on the morning of the first day in heat and was found to be 32 grams/min. At the beginning of the fourth period he fainted as he was walking up the 3 steps to the treadmill platform. Five subsequent determinations of the rate of sweating on Wa when he was able to resume work averaged 12.8 grams/min. It should be noted that Wa had a milder though similar episode in the afternoon of the same day with a normal rate of sweating.

Electrocardiograms were obtained on all men during the control period and at the end of the experiment. Records were also obtained on all men suffering from heat exhaustion. In no case did the low plasma chloride influence the electrocardiographic pattern.

The heat exhaustion encountered in this Laboratory differed from the "dehydration exhaustion," reported by Adolph (13) on subjects working at high temperatures without water, in that nausea and vomiting were a prominent feature, occurring in 60 per cent of our cases while Adolph reported 35 cases of dehydration exhaustion without either nausea or vomiting. McCance (14) has reported that nausea and vomiting occur in uncomplicated salt deficiency.

In a previous report (4) it has been shown that heat exhaustion is associated with the failure of men to acclimatize (as measured by the pulse rate) and with a poor cardiovascular postural adjustment test in the evening preceding the actual development of symptoms. It was also pointed out that food (with salt), water and rest (subjects remaining in the heat) are the only measures that are ordinarily necessary to restore to activity the men who develop heat prostration.

DISCUSSION. The evidence presented here indicates that men working in the heat, sweating 5 to 8 liters a day do not require more than 15 to 17 grams of salt a day. The total 24-hour sweat volumes are similar to those measured in the desert by Dill *et al.* (17), in mines by Moss (2) and recently by ourselves in desert maneuvers. Salt requirements for tropical conditions also may be estimated on the basis of these experiments since Lee *et al.* (15) have shown that for a given effective temperature (Houghten and Yaglogou, 16) the salt loss during work is less in the hot wet conditions than in the hot dry conditions. Thus it may be expected that men sweating 5 to 8 liters in the tropics will not require more than 15 to 17 grams of NaCl a day.

It is apparent that the salt requirement may be best judged by the physiological response of the individual. For this reason, we wish to emphasize the results of series III in which no significant physiological advantage was gained by increasing the NaCl consumption from 15 to 30 grams a day during work in the heat.

The relationship of cardiovascular function to heat exhaustion has been recognized by other investigators (Weiner, 18; Keeton *et al.*, 19; Ferris *et al.*, 20). A number of observers, who were primarily concerned with the study of heat cramps, have noted without giving data that when adequate NaCl is supplied to miners (Moss, 2) or steel workers (Dill *et al.*, 3) subjective fatigue is reduced and the incidence of heat exhaustion lowered. Clear evidence is submitted here to show that a salt deficit imposes a severe strain, in addition to the task of heat regulation, on the cardiovascular system of men performing work in high environ-

mental temperature and that this additional burden will limit the amount of work performed and in some cases lead to the development of heat exhaustion. The cause of this effect appears to be a contracted circulating blood volume. The extent of the reduction of the blood volume in these experiments may be estimated from the NaCl loss. The estimated NaCl loss of the low NaCl group for the first 24 hours was 8.39 grams. If the chloride all came from the extracellular fluid, this would represent 1.74 liters. This is 10.3 per cent of 16.87 liters, the average extracellular fluid found by Forbes, Dill and Hall (21) in 10 men. This change is the same order of magnitude as the per cent change in the refractive index increment of the plasma (15.2 per cent), a figure which may be taken as roughly proportional to the blood volume change.

Heat exhaustion appears to be circulatory failure or impending circulatory failure during activity. The subject cannot perform continuous physical work, indeed he may faint while attempting it, but if he ceases physical activity and lies down the capacity of his cardiovascular system is adequate to meet the requirements of resting metabolism. Circulatory failure can be brought on by placing excessive demands on the heat regulating mechanism (18). The more common situation appears to result from the integrated effects of a contracting blood volume and the demands of heat regulation for circulation through the skin. The blood volume may be reduced by failure to drink enough water to keep up with the water lost by sweating (13) or by failure to consume enough chloride to replace the salt lost in sweat production (present results).

Talbott (1) has maintained that the lowering of sodium and chloride ions in serum is the principal etiological factor in the pathogenesis of heat cramps. Both the concentration of chloride in plasma and the total chloride loss of the subjects on the low NaCl diet were well within the range reported as being characteristic of heat cramps. Talbott and Mickelsen (22) reported 5 cases of heat cramps with an average serum chloride of 96.9 M.E./liter on admission. Eight subjects of the 20 studied on the low NaCl diet had plasma chloride concentrations of less than 100 M.E./liter, the average being 94.6 and the lowest 89 M.E./liter. None of these men had real heat cramps but 5 of them suffered heat exhaustion. One man, C. E., complained of slight twitchings in the right ankle and hip but this could hardly be termed true heat cramps. These observations serve to emphasize that no one factor accounts for the development of true heat cramps (3). Dill *et al.* (3) have suggested that the severity of heat cramps depends both on the intensity of the work and the degree of salt loss. It is probable that the intensity of work in these experiments was not as great as that encountered for brief periods in steel mills and mines although the total work output cannot be much inferior judging from pulse rates. In any case it is clear that NaCl loss *per se* and the resulting reduction of plasma chlorides will not necessarily result in heat cramps but will lead in many cases to heat exhaustion.

SUMMARY

The effects of 3 levels of NaCl intake on cardiovascular functions were studied in 49 "normal" men in work and rest during exposure to hot, dry conditions.

The salt intakes were, in grams NaCl per 24 hours, 6 ± 2 ("low"), 15 ± 2 ("moderate") and 30 ± 2 ("high"). Day temperature was 120°F. dry bulb, wet bulb of 85°F., night 85° to 95°F. dry bulb, 65° to 75° wet bulb. Control studies were at 80°F. dry, 65°F. wet bulb. Diet, exercise and other conditions were rigidly standardized. Water was allowed *ad lib*. Periods in the heat ranged from 2 to 8 days each.

Pulse rates, rectal temperature, sweat composition and rate of sweating were studied in work and in rest. Blood ptosis tests were made. Sweat production ranged from 5 to 8 liters per day.

No advantage in any of the variables measured was demonstrated for men on a "high" daily intake of 30 grams NaCl as compared to the "moderate" (15 grams) intake.

Men maintained on a "low" (6 grams) intake of salt had higher pulse rates and rectal temperatures in work than men on a "moderate" salt intake. The deleterious effect of the "low" salt intake was also reflected in poorer postural cardiovascular adjustment. The men on the "low" salt intake lost more than twice as much body weight, drank less water and sweated less than the men on the "moderate" salt intake.

The "low" salt intake resulted in an average net deficit of 13 grams NaCl for 3 days in the heat. The men on the "moderate" salt intake appeared to be in NaCl balance after 3 days in the heat.

Heat exhaustion and prostration, characterized by nausea, vomiting, tachycardia, hypotension, vertigo, dehydration and collapse, occurred in 25 per cent of the men on the "low" salt intake and in only 2.5 per cent of the men on the "moderate" salt intake. Rest in the heat, food (with salt) and water sufficed to restore all these men so that they could continue work in the heat in 8 to 24 hours.

Although pronounced hypochloremia was observed in many instances heat cramps did not occur.

There was little or no relation between the concentration of chloride in the sweat and the rectal temperature.

It is concluded that heat exhaustion and ability to work in the heat are almost wholly dependent on cardiovascular function and that a moderate salt intake is more important to preserve this function than to prevent heat cramps. Hypochloremia is not the only factor in heat cramps.

It is further concluded that the NaCl requirement of unacclimatized men who are sweating 5 to 8 liters a day is not greater than 13 to 17 grams daily. An increase in salt intake above this level results in increased loss of salt and water in the urine with no apparent advantage.

Acknowledgments. Completion of this work would not have been possible without the careful attention to detail and willing help of Miss Angie Mae Sturgeon, Head Technologist, Miss Evelyn Pearson, Dietitian, Mrs. Nedra Foster, Assistant Dietitian and Mr. Howard Condiff, Assistant Chemist. We also wish to thank Dr. Josef Brozek for assistance in the statistical analysis of the data.

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EFFECTS OF PURIFIED ANTERO-PITUITARY HORMONES ON THE CARBOHYDRATE STORES OF HYPOPHYSECTOMIZED RATS¹

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It has been recognized for some time that antero-pituitary hormones are needed to enable hypophysectomized rats to withstand fasting periods without marked loss of carbohydrate stores. It has been demonstrated that adrenal cortical steroids (3, 16) or a crude adrenocorticotrophic hormone (4) will preserve or increase blood sugar, liver glycogen and muscle glycogen levels of fasted hypophysectomized rats. If a crude anterior lobe extract is given throughout the fasting period, complete maintenance of muscle glycogen levels may be produced without effects on blood sugar or liver glycogen. This effect may also be obtained in the absence of both adrenals (11). The pituitary principle which produces this effect has not been identified with any of the known anterior lobe hormones and has been termed the glycostatic hormone (1, 2).

By employing the most purified anterior hypophyseal hormone preparations now available it was hoped that a long deferred answer might now be given the question "Is myoglycostatic activity a property of one or of several of the six known antero-pituitary hormones?" In the course of these studies concordant observations were frequently made on the hepatic glycogen and blood sugar.

EXPERIMENTAL CONDITIONS, METHODS AND MATERIALS. In most of these experiments, conditions were similar to those proposed by Russell for the assay of the myoglycostatic hormone (2). Male rats, hypophysectomized when 2-3 months old, were used 2-4 weeks postoperative. In part of the experiments, immature females, operated upon at the age of 26-28 days were employed 5-10 days postoperative, but occasionally as long as 3.5 months after the operation. In each experiment only animals of the same age and postoperative period were used, generally in groups of 5-8 rats. All rats were fasted for a 24 hour period, started at 1 p.m. and received the hormone intraperitoneally in three doses at 24, 10-12, and 4 hours before sacrifice. During longer injection periods the hormone was given once daily save on the last day when the schedule mentioned above was followed. Thus in a series of experiments, rats were treated for 3-4 days, starting two weeks postoperative, in another series for 9-21 days, starting on the day following operation.

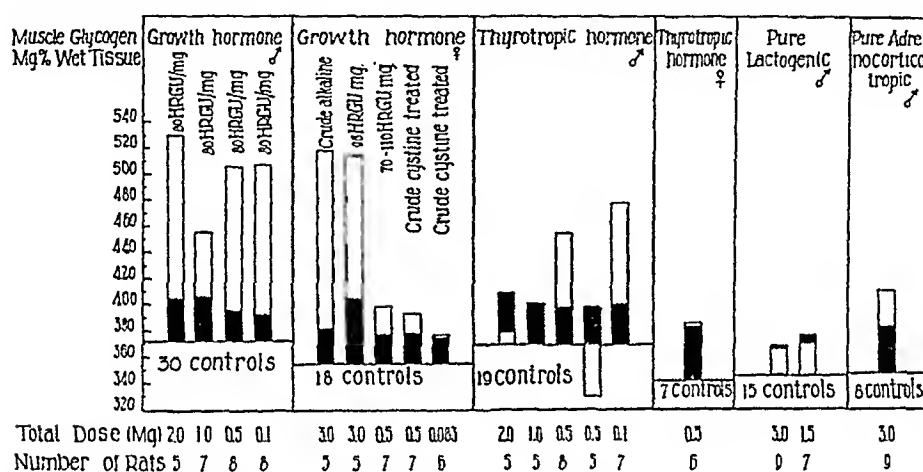
The hormone preparations while in general similar to those used in previous metabolic studies (5, 6, 7), embraced two highly purified substances—the adrenocorticotrophic and lactogenic hormones. The thyrotropic preparation contained

¹ Aided by grants from the Research Board of the University of California and the Rockefeller Foundation, New York City.

slight amounts of the interstitial cell stimulating hormone and an uncertain amount of growth hormone.²

All rats were anesthetized with sodium amytal for the collection of blood and tissue samples. The liver and muscle glycogens were determined by a minor modification of the Cori method (8) used in this laboratory for several years. This consists in proceeding after the acid hydrolysis without filtration. This was demonstrated by control experiments to be non-essential. The glucose determinations were performed by the method of Benedict (9). When an ignited molybdic anhydride was used in preparing the color reagent, a very stable color was obtained, reaching a color maximum in 30 minutes which changed less than 2 per cent during 8 hours. The Sheard-Sanford photoelectric colorimeter was used. Blood was deproteinized by the copper-tungstate method of Somogyi (10).

DISCUSSION OF RESULTS. The data are presented in graphic form to show the



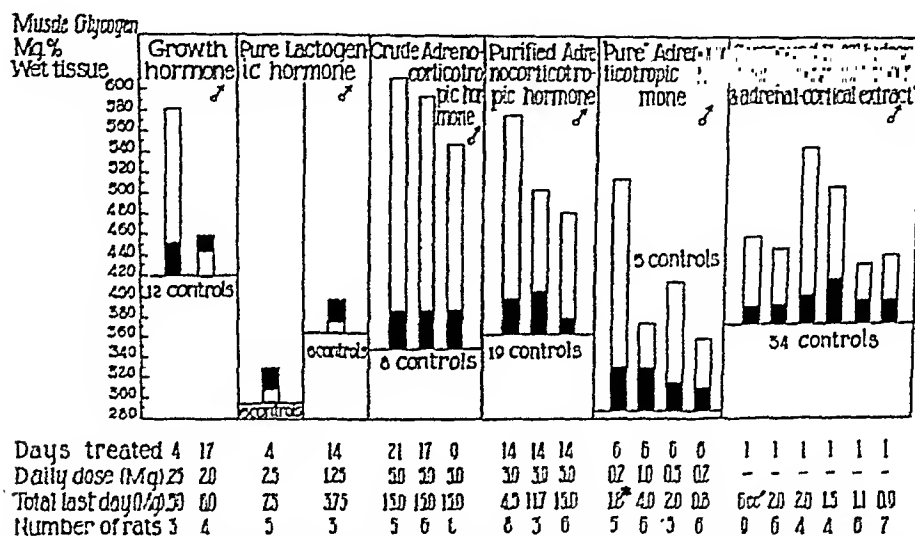
Graph I. The myoglyceostatic effect of purified anteropituitary hormones administered during fasting in three divided doses at the 24th, 10-12th, and 4th hours before sacrifice of hypophysectomized rats.

increase of the experimental groups over their respective controls in graphs I and II; the black areas indicate the standard deviation³ of the difference and an increase of two times this was considered significant. When Russell (1, 2) studied and compared the ability of various pituitary fractions to maintain muscle glycogen in fasted hypophysectomized rats, she found growth promoting extracts always to be potent in this respect. Since then methods have been developed for a better separation of the growth principle from the other known hormones. When such improved preparations were tested for myoglyceostatic

² We desire to thank heartily Dr. Walter Marx for many samples of a highly purified and potent growth hormone, Drs. William R. Lyons and C. H. Li for completely purified lactogenic hormone, Dr. C. H. Li for completely purified adrenocorticotrophic hormone and Jane Fraenkel-Conrat for thyrotropic preparations which had been purified to a fargoeing extent. We especially desire to thank Dr. Heinz Fraenkel-Conrat for the suggestion that these studies be undertaken and for his kindness throughout their execution and formulation.

³ Standard deviation of the difference between the mean of the controls and the mean of the experimental groups.

activity, they were still found to be effective. About ten different preparations of growth hormone were given to a total of 64 rats and all groups treated with the hormone showed increased muscle glycogens when compared with the controls (graph I). One milligram of various preparations caused increases of 50-90 mgm. per cent. In one experiment as little as 0.1 mgm. of a highly purified growth hormone preparation caused statistically significant maintenance, but higher doses of this preparation were not appreciably more effective (graph I). This is not very surprising in view of Russell's finding of a very narrow range in which a clear-cut dose-response relationship could be observed (2). Treatment with the hormone for 3 or 4 days preceding the fast appeared no more effective than the usual one day injection period, while after 17 days of hormone treatment no appreciable effect was noted (graph II). (Confirmation of L. L. Bennett, 11.)



Graph II. The myoglyceostatic effect of purified anteropituitary hormones administered daily, and in three divided doses at the 24th, 10-12th, and 4th hours before sacrifice, on the last day of fasting.

* Injected nine times, at two hour intervals, during the last day of treatment (other ACTH groups received four injections, at 3 hour intervals, during the last day of treatment).

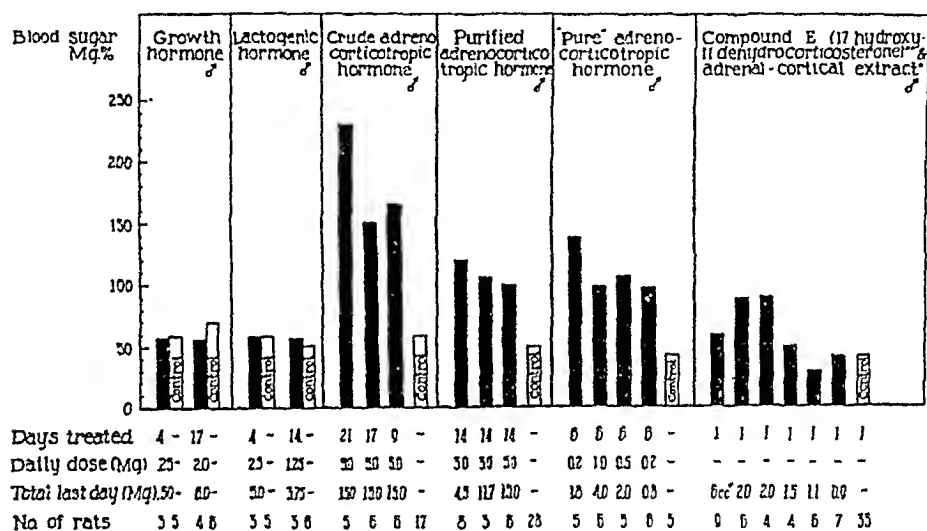
In contradistinction to the effectiveness of growth hormone preparations in maintaining muscle glycogen, they did not maintain blood sugar (graph III) or liver glycogen under the same conditions.

Thyrotropic extracts were generally found to be rich in glyceostatic activity in older studies (2). Forty-four hypophysectomized rats were therefore injected with a number of purified thyrotropic preparations. Results indicated that there was no statistically significant maintenance of muscle glycogen in 5 out of 7 experiments. The two groups in which maintenance did occur might be accounted for by the contamination with growth hormone.

Pure adrenocorticotrophic hormone (ACTH) (12) was tested at one level in the usual test (graph I). When 3 mgm. were given, muscle glycogens were found 65 mgm. per cent above controls, the difference not being significant.

The previous experiments with less pure adrenocorticotrophic, gave similar small increases. In a considerably greater number of experiments, however, ACTH was given for periods ranging from three to twenty-one days, starting the treatment in many experiments on the day following the operation and in others from two weeks to three and a half months postoperative (graph II). In all these experiments muscle glycogens were maintained. Blood sugar determinations in these groups paralleled muscle glycogen values strikingly, the blood sugars ranging up to 233 mgm. per cent in the treated groups (graph III). The highest muscle and blood carbohydrate values were obtained with a crude ACTH. In contradistinction to the finding of Bennett (4), liver glycogen though found to be slightly increased in these experiments was never super-normal.

In view of the general belief that the hormonal control of blood sugar and liver glycogen depends on the adrenal and that these values are generally correlated,



Graph III. The blood sugar levels of hypophysectomized rats given anteropituitary hormones daily and three times during the last day while fasting.

while muscle glycogen is only secondarily influenced by this gland (2, 4, 5, 7) many control experiments were performed to verify the surprising findings here reported; namely, that stimulation of the adrenal had only minor effect on liver glycogen, while causing hyperglycemia and increased muscle glycogen values. When the analytical technique was tested repeatedly by the determination of known amounts of hog liver glycogen added to rat livers, recovery was regularly found to be above 87 and generally close to 92 per cent, if the following precautions were observed: 1, the tubes containing 30 per cent KOH were placed into a boiling water bath immediately after the addition of the freshly extirpated livers; 2, sugar determinations were carried out immediately after acid hydrolysis of the liver glycogen. In some of the earlier experiments, before the importance of these precautions was realized, one secured approximately 0.10 per cent lower liver glycogen values. However, experimental and control rats were treated alike in each case and since recent experiments in which this error was avoided

confirmed the findings of the earlier ones, no significance can be attributed to this factor in the interpretation of the results.

It appeared of importance to re-study the effect of adrenal extracts and crystalline steroids on the glycogen and sugar stores of hypophysectomized rats. Six groups of rats were treated with 17-hydroxy-dehydrocorticosterone (compound E of Kendall⁴) during the 24 hour fast with doses ranging from 0.4 mgm. to 2.0 mgm. (graph II). Muscle glycogen values were found increased by all but the lowest level of the hormone, the effect being significant after the administration of more than 1.1 mgm. Blood sugars were also well maintained by 2 mgm. of compound E, with doubtful effects at lower doses (graph III). Liver glycogen again was found low in all experiments never exceeding 0.2 per cent, while the controls were generally about 0.13 per cent. Similar effects were produced by 6 cc. of Upjohn Cortical Extract. Thus cortical extract and compound E were found to act quite similar to ACT in our rats, causing appreciable increases in muscle but not in liver glycogen; in regard to blood sugar the pituitary hormone caused hyperglycemia while the steroid at the dosages used only maintained blood sugars at levels similar to those of normal fasted rats. However, it appears quite possible that the mode of administration and the speed of action of the steroids may here be an important factor and that with continuous administration in larger amounts these compounds would produce blood sugar and liver glycogen increases similar to those reported by others for adrenal steroids (3, 16, 17). It is especially noteworthy that the low doses used significantly increased muscle glycogen.

Myoglycostatic studies have been reported for growth, thyrotropic and adrenocorticotropic hormones. The remaining chemically characterized pituitary hormone of significance is the lactogenic hormone. This hormone can be prepared in a state of uniform potency and our various physico-chemical studies indicate that it is a pure protein (13, 14). In the two experiments presented (graph II) in which it was administered to hypophysectomized rats for more than 24 hours the adrenals did not show an increase in weight and no histological stimulation was evident. When the hormone was given only during the fasting period at dose levels of 1.5 and 3.0 mgm. no significant increases in muscle glycogen were observed (graph I). When it was given for 4 and 14 days to two other experimental groups at levels of 2.5 and 1.25 mgm. daily with three times this dose during the fasting period there likewise was no significant increase in the muscle glycogen (graph II). Throughout the experiments with lactogenic hormone liver glycogen and blood sugars were uniformly low.

The data presented in graph I show that no greater effects were obtained with a purified cysteine-treated growth hormone preparation than with a crude alkaline extract. Euglobulin and pseudoglobulin fractions of a crude extract were found active at 1 mgm. while the albumin fraction showed no activity at 2 mgm. The fact that purified hormone preparations were generally no more active than crude extracts indicated the possibility that the latter might owe their activity to a synergism of some of their component hormones. Therefore a considerable number of hormone combinations were tried in search for this

⁴ We desire to thank Dr. E. C. Kendall heartily for this valuable substance.

synergism. When growth hormone was given together with ACTH and lactogenic hormone for one day, the effect was no greater than that produced by the growth hormone alone. When the same combination was given for 3 days, it caused the highest muscle glycogen values (230 mgm. per cent above controls), but the effect was less than additive. Also upon combination of growth hormone with thyrotropic or with 17-hydroxy-dehydrocorticosterone (compound E) no synergism was noted.

CONCLUSIONS. Russell found myoglycostatic activity to varying extent in all anterior pituitary extracts tested with the exception of the albumins (growth discard fraction) and a lactogenic preparation (2). Using the same technique, i.e., the 24 hour fasting period with equal amounts of hormone at 24th, 10th to 12th and 4 hours before sacrifice, we find purified growth hormone alone to show pronounced effects at low doses. Russell hesitated to identify the myoglycostatic hormone with the growth hormone, because of the glycostatic activity of other hormone fractions, low in growth activity, and because of the fact that the purification of the growth hormone was not paralleled by an increase in myoglycostatic potency, when compared to crude extracts. The first instance may have been confusing because of adrenotropic hormone contamination inhibiting the growth activity of the extracts (15), and thereby producing extracts apparently low in growth potency. Secondly, we have confirmed the failure of growth preparations to show marked increases in myoglycostatic potency during purification. However, the fact that growth hormone has since been nearly freed from several known hormones (ICSH, FSH, lactogenic, adrenocorticotropic, and thyrotropic) without losing myoglycostatic activity at low dose levels (0.1 to 1 mgm.) indicates that one or both of the two components present by electrophoretic analysis of purified growth fractions might be the myoglycostatic agent, or agents.

The time relationships of the muscle glycogen increases presented in this paper are of the greatest importance.

a. Growth hormone is most effective when given for 1-3 days. It becomes ineffective in maintaining muscle glycogen when given for 2-3 weeks (4).

b. Adrenocorticotropic hormone is only slightly effective or inactive when 3 injections are given during the 24 hour fast; but it is very effective when given daily for one to two weeks. This suggests that dissimilar mechanisms are responsible for muscle glycogen storage by growth and adrenocorticotropic hormones. Maintenance of adrenal cortical size and secretion by adrenocorticotropic hormone gives increased muscle glycogen levels in the hypophysectomized rat (graph II) which are similar to those obtained with 17 hydroxy-11-dehydrocorticosterone (Kendall's Compound E). Similar increases have been previously reported for cortical extract and corticosterone (Kendall's Compound B) by Russell and Craig (3) and Long and Katzin (16). Russell and Craig (3) obtained supernormal muscle glycogen levels when adrenal steroid (Compound B) and "glycostatic" extract were administered simultaneously to hypophysectomized rats.

Two factors are probably instrumental in the failure to obtain supernormal

liver glycogen in fasted hypophysectomized rats given compound E, or cortical extract reported in graph II.

a. The hormone or extract was not given in small hourly doses. Reineke and Kendall (17) have recently emphasized the importance of hourly administration of potent extracts to obtain liver glycogen storage in fasting adrenalectomized rats.

b. The amount of cortical extract just sufficient for the maintenance of normal carbohydrate levels in adrenalectomized rats is without effect in the absence of the hypophysis (Bennett, 18; Grollman, 19). Russell (20) presents an excellent analysis of the interplay of glyconeogenetic action of adrenal cortex hormones and crude anteropituitary extract effects upon glucose utilization rate in the eviscerated rat. The hypophysectomized rat oxidized sugar at a much greater rate than the normal or adrenalectomized rat.

In recent experiments (21) pure adrenocorticotrophic hormone administered to hypophysectomized rats fasting during low pressure treatment has not increased their liver glycogen to levels found in anoxic rats with intact hypophyses. In these animals, the adrenals were larger than normal, and blood sugar levels, and muscle glycogen markedly increased by the adrenocorticotrophic administration. Bennett (4) using an impure adrenocorticotrophic hormone at high dose levels obtained supernormal liver glycogen levels in fasted hypophysectomized rats. Experiments are planned to determine what anteropituitary hormone or hormones are necessary for administration with pure adrenocorticotrophic hormone to give supernormal liver glycogens in fasting hypophysectomized rats. Those anteropituitary fractions high in myoglycstatic and growth hormone action are highly effective in depressing the respiratory quotient of glucose fed rats (22, 23, 24). This action if it reduces glucose oxidation in the periphery might conceivably act with adrenotropic hormone to increase the liver glycogen during fasting to supernormal levels.

SUMMARY

1. Myoglycstatic activity is greatest in crude extracts of the anterior hypophysis and in highly purified growth promoting extracts.

2. Thyrotropic extracts known to be impure had activities no greater than to be expected from possible growth hormone contamination.

3. Adrenocorticotrophic hormone showed slight or no activity during the one day test in which growth hormone preparations were highly effective. However, when through longer administration of adrenocorticotrophic hormone, the adrenal gland becomes normal or hypertrophied, muscle glycogen and hepatic glycogen are maintained by it during fasting.

4. Pure lactogenic hormone is ineffective in the one day myoglycstatic test or when administered for 4 or 14 days.

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THE EFFECT OF REPEATED DETERMINATIONS ON THE BASAL METABOLISM OF CHILDREN¹

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Prediction standards for the basal metabolism of children from 2 to 15 years old, inclusive, have been established by the Child Research Council of Denver on the basis of 1,007 determinations on 70 healthy boys and 718 determinations on 57 healthy girls in this age range. These values have been recently reported by Lewis, Duval and Iliff (1). The study has involved repeated determinations of basal metabolism on each child, usually at intervals of from 3 to 5 months. The validity of basing standards for the prediction of basal metabolism on repeated observations on the same individuals has been questioned on the ground that the element of experience through repetition of the same procedure may enter into such determinations and result in lower average values than would be found if only the first satisfactory determination on any individual were used. The purpose of this report is to evaluate the effect of such repeated determinations of the basal metabolism of children.

Review of literature. Boothby, Berkson and Dunn (2) and Eaton (3) have contended that standards of basal metabolism for clinical use should be based solely on the first satisfactory observation on any individual, since this procedure more nearly represents that found in clinical practice. This contention does not seem entirely justified. While it is true that many individuals who are tested for clinical reasons and give values within the normal range on their first satisfactory determination of basal metabolism are not subsequently retested, it is equally true that patients whose basal metabolic rates are found to lie outside the normal range are frequently subjected to repeated testing in order that the clinician may follow the results of treatment. Thus, if the repetition of determinations affected the level of results, the use of standards based solely on first satisfactory determinations would be unwarranted when repeated observations are required.

A number of workers have recognized the possible effect of experience by the

¹ A preliminary report of this study was made before the Rocky Mountain Section of the Society for Experimental Biology and Medicine at Denver in March, 1943.

subject on the procurement of satisfactory determinations of basal metabolism and have used one or more preliminary practice periods, either on the same day or on the day before the actual experimental period was begun. In several studies of basal metabolism that necessitated repeated observations on the same children, a comparison of the first few determinations has been made to see if experience has had any effect on the results obtained. Shock (4) demonstrated that, provided there is a preliminary testing period the results of which are discarded, the reliability of metabolism measurements is not significantly altered by repeated testing. Bierring (5), noting that the first two determinations on the boys of his study were very seldom satisfactory, advocated a preliminary practice period to obtain reliable results. MacLeod (6) was able to demonstrate no uniform or marked effect of experience on the results of her study in which repeated determinations of basal metabolism were made. Webster, Harrington and Wright (7) found that 11 of 21 children studied gave results on the first observation that were not appreciably higher than those on subsequent tests, while on the second day eight of the remaining children reached a relatively constant level which was maintained thereafter.

EXPERIMENTAL PROCEDURE. The data to be discussed in this report have been taken from the studies of Lewis, Kinsman and Iliff (8) and of Lewis, Duval and Iliff (9). The subjects are members of the group of healthy children who are being studied regularly by the Child Research Council. Determinations of basal metabolism with the open circuit chamber method have been made, usually at intervals of from 3 to 5 months, by the technic described earlier (8). The generally accepted conditions for basal metabolism have been strictly followed, and only those results which were judged to be satisfactory at the time of the determination have been reported. Only observations during which the subjects were awake but relaxed and free from muscular activity have been considered satisfactory.

The method of treating the data to determine if the basal metabolism is lower in general on experienced than on inexperienced subjects has been to compare the first determination on each individual with those obtained on subsequent days. However, since the basal metabolism expressed as calories per hour per square meter varies with age, absolute values cannot be made the basis of comparison of determinations at one age with subsequent determinations at an older age. Accordingly, the comparison has been made on the basis of the percentage deviations of the observed values from the standard values of Lewis, Duval and Iliff (1).

RESULTS AND DISCUSSION. The effect of repeated testing would be particularly evident for those children who have given satisfactory results on each of the first few days that determinations have been attempted, since these subjects would have had no other opportunities to become accustomed to the procedure. Satisfactory results have been obtained on the first two determinations attempted on 26 children whose mean age at the time of the first determination was 5 years 5 months within a range of from 2 to 10 years. Figure 1, graph A, represents the frequency distribution of the differences between the percentage deviations

from the standard when the second is compared with the first determination in these instances. The mean difference is only -0.3 , which cannot be considered significant. However, since this histogram departs somewhat from the normal probability curve, the analysis has been carried further. Figure 1, graph B, compares the percentage deviations from the standard on the third actual determinations with those on the first actual determinations on 21 children and reveals a normal distribution for these data. In this instance the mean difference

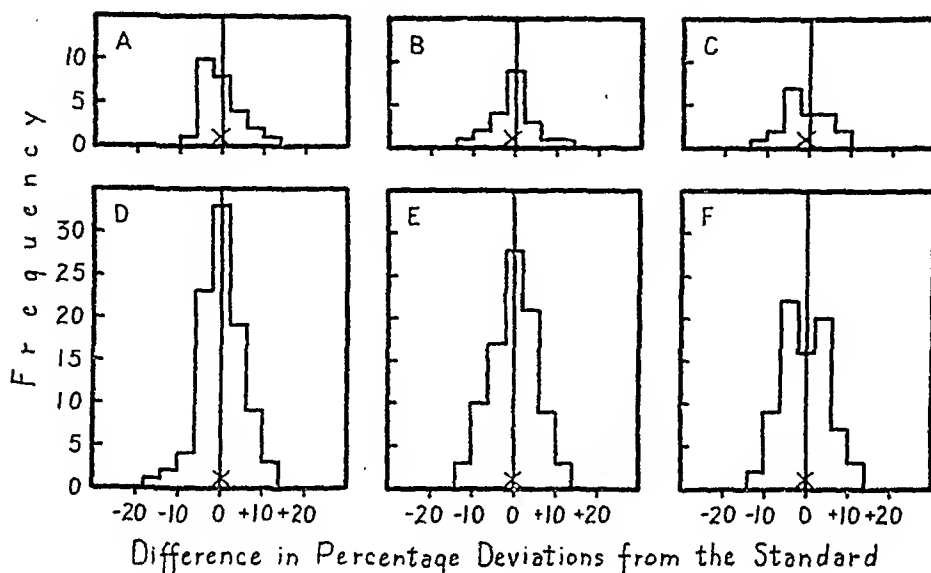


Fig. 1. Histograms showing the frequency distributions of the differences between the results of repeated determinations of basal metabolism expressed as percentage deviation from the standard. In each graph the mean difference is indicated by X.

Graph A represents the variation of second actual from first actual determinations on 26 children.

Graph B represents the variation of third actual from first actual determinations on 21 children.

Graph C represents the variation of fourth actual from first actual determinations on 20 children.

Graph D represents the variation of second from first satisfactory determinations on 94 children.

Graph E represents the variation of third from first satisfactory determinations on 91 children.

Graph F represents the variation of fourth from first satisfactory determinations on 79 children.

is -0.6 . Similarly, figure 1, graph C, which also shows a symmetrical distribution of data, compares the results on the fourth actual determinations with those on the first actual determinations on 20 children. This comparison reveals a mean difference of -0.8 . That none of the mean differences found are large enough to be significant may be seen from their respective standard deviations in table 1. In order to calculate the standard deviations with accuracy, it has been necessary to assign an arbitrary value of 100 to the zero point on each histogram in figure 1. In each instance the standard deviation is relatively so

great as to render insignificant the observed mean difference in percentage deviation from the standard.

As a further test of the significance of the differences found between the results of the first and subsequent actual determinations, the chi-square technic has been used on the frequencies with which the individual results in each group fall above or below the mean for the combined groups. These values for chi square and their respective probabilities, which are also given in table 1, reveal that all of the observed differences may well have arisen from chance selection alone. Thus, no significant differences, such as would be found if experience affected the level of the basal metabolism, can be demonstrated.

TABLE 1

Statistical analysis of the results obtained on repeated determinations of basal metabolism

COMPARISON OF	MEAN DIFFERENCE BETWEEN PERCENTAGE DEVIATIONS FROM THE STANDARD	THE MEAN DIFFERENCE WITH ARBITRARY ADDITION OF $100 \pm$ STANDARD DEVIATION THEREOF	CHI SQUARE*	PROBA- BILITY†
Second with first actual determinations...	-0.3	99.7 ± 4.3	0.31	0.58
Third with first actual determinations....	-0.6	99.4 ± 5.2	0.39	0.53
Fourth with first actual determinations...	-0.8	99.2 ± 5.4	0.40	0.53
Second with first satisfactory determina- tions.....	+0.2	100.2 ± 4.9	0.34	0.56
Third with first satisfactory determina- tions.....	± 0.0	100.0 ± 5.2	0.09	0.76
Fourth with first satisfactory determina- tions.....	± 0.0	100.0 ± 5.6	0.92	0.34

* The value for chi square has been calculated from the frequencies with which the results of each group, expressed as percentage deviation from the standard, fall above or below the mean of the combined groups.

† Since the probability of occurrence, as obtained from the value for chi square, is greater than 0.05 in each instance, chance selection will account for all of the observed differences between the first and subsequent determinations.

Since all children are not so co-operative that satisfactory results are obtained on the first day determinations are attempted, it is important to consider the results of the first, second, third and fourth satisfactory determinations on each child without regard to the actual number of attempts to make determinations on that child. Values suitable for such comparison are available for first and second satisfactory determinations on 94 children; for first and third satisfactory determinations on 91 children; and for first and fourth satisfactory determinations on 79 children. Figure 1, graphs D, E and F, show the frequency distributions of the differences between the percentage deviations from the standard when the second, third and fourth satisfactory determinations, respectively, are compared with the first satisfactory determinations on these children. The mean differences found are $+0.2$, ± 0 and ± 0 ; the distribution in all three cases approximates the normal probability curve; and application of the chi-square

technic to these data shows (table 1) no differences which could not have arisen from chance selection. Hence, it may be concluded that neither the second, the third nor the fourth satisfactory determinations on these children are lower as the result of practice than the first satisfactory determinations. However, it must be remembered that several attempts may be necessary before a satisfactory determination is obtained. Of the 94 subjects discussed above, 33 children or 35 per cent gave satisfactory results on the first determination, 52 children or 55 per cent had given satisfactory results by the second determination, and 75 children or 80 per cent had given satisfactory results by the third determination. In one instance seven determinations were required before a satisfactory result was obtained.

In none of the instances depicted in figure 1 and table 1 are the differences between the percentage deviations from the standard consistent enough to imply an effect from repetition of determinations that might invalidate standards established on this basis. It must be remembered, however, that all of the determinations upon which this conclusion is based had passed certain definite criteria. No determination can be considered satisfactory from the standpoint of co-operation of the subject unless the child refrains from muscular activity and is relaxed but awake. If these conditions are met by any individual, he may be considered a satisfactory subject, and subsequent determinations may be expected to check within the range of intra-individual variation. The readiness with which satisfactory determinations may be obtained varies considerably with different children. A preliminary testing period is necessary only in so far as it is required to obtain co-operation of the child to the end that satisfactory results may be obtained on him with ease. The period required to obtain such co-operation varies markedly from a few minutes on one day to repeated attempts on several days and is dependent both upon the temperament of the child and the readiness with which he understands the necessary directions. In general, a younger child will require greater persuasion to remain perfectly quiet and at ease than will an older one. Prediction standards for basal metabolism established by a longitudinal study such as that being carried out by the Child Research Council may be used to evaluate the results of any satisfactory determination on a child regardless of whether he has had only the one determination or many other previous tests.

SUMMARY

1. Because the suggestion has appeared in the literature that standards for basal metabolism based upon repeated determinations on the same individuals are lower than those computed solely from first satisfactory determinations, the effect of repeated testing on the level of the basal metabolism has been considered.

2. Comparison of the results of the first satisfactory determination of basal metabolism with those of the second, third and fourth satisfactory determinations on children who varied in age from 2 to 10 years reveals no significant difference which can be attributed to experience. Of 94 subjects, 35 per cent gave satis-

factory results on the first determination, and 55 and 80 per cent, respectively, had given satisfactory results by the second and third determinations.

3. Analysis of the results of the first determinations on those children who gave satisfactory results on the first day that observations were attempted reveals no significant difference from the results of the second, third and fourth actual determinations.

4. A preliminary testing period may or may not be necessary to procure satisfactory results in the determination of the basal metabolism of children. Accordingly, no practice period is prescribed, but the first determination in which the child refrains from muscular activity and is relaxed but awake is accepted as satisfactory. Experience by the subject tends as a rule to increase the ease with which a satisfactory determination may be obtained.

5. The prediction standards for the basal metabolism of children from 2 to 15 years old, inclusive, that have been established by the longitudinal study of the Child Research Council (1) may be used to evaluate the results of any satisfactory determination on a child regardless of his familiarity with the procedure.

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THE EFFECT OF DAMAGE TO THE TRACHEAL MUCOSA UPON THE DRAINAGE OF RESPIRATORY TRACT FLUID

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The secretions and fluids in the respiratory airway, which will be referred to as R.T.F. or Respiratory Tract Fluid, are brought up the tracheo-bronchial tree, or otherwise eliminated, by several mechanisms which have been listed (1) as the ciliary mechanism, cough, milking-like contractions of the muscles of Reissersson, the churning movements of breathing and reabsorption into the lymphatics and veins of the lung. The present report is concerned chiefly with the part played by ciliary movements. Ciliated columnar or cuboidal epithelial cells extend upward to the nose from near or about the junction of the terminal and respiratory bronchioles and are theoretically capable of sweeping along R.T.F. at the rate of 1 to 5 feet per hour, the rate being most rapid in the trachea (2).

There exists a difference of opinion as to the part actually played by the ciliary carpet in the drainage of R.T.F. The Swiss investigator, Gordonoff (2), concludes, "die Flimmerbewegung für den Expektationsvorgang von geringer Bedeutung ist," while the French scientist, Policard (3), more cautiously notes, "la machine ciliare bronchique n'a pas une grosse réserve de puissance." British and American authors have attributed more importance to ciliary drainage. Robertson (2) considers it to be the principal mechanism of drainage of the lung above the respiratory bronchioles while Gunn (4) goes as far as to state that under normal conditions ciliary drainage alone is responsible for removal of bronchial secretions.

In none of the papers reviewed by the above authors was the output of R.T.F. actually measured under conditions of normal and impaired ciliary function so that there is no direct information on the ciliary drainage of R.T.F. In the experiments to be reported below, the output of R.T.F. was measured in rabbits and cats with a normal tracheo-bronchial mucosa and with the tracheo-bronchial mucosa damaged by the inhalation of ammonia gas and live steam.

The method used to collect R.T.F. was that of Perry and Boyd (5) with later improvements described by Boyd, Jackson and Roum (6). Briefly, it consists in ligating a T cannula into the trachea of a methanized animal, collecting the R.T.F. in a graduated tube and conditioning the inhaled air to body temperature and approximately 100 per cent relative humidity, all of the exposed parts of the apparatus containing inhaled or exhaled air being thoroughly insulated. The output of R.T.F. was measured at intervals of half an hour and the volume was expressed as ml. of R.T.F. per kilo body weight per 24 hours.

The inhalation of ammonia gas. Thirty-two rabbits and 6 cats were exposed for 1 hour in a static gassing chamber of 400 litre capacity to a concentration of 3.5 to 8.5 mgm. of NH_3 per litre of air. This concentration of ammonia is

described by Ficklen (7) as "rapidly fatal." Actually the animals lived almost as long as did the controls not gassed with ammonia, both sets of animals being urethanized and arranged for collection of R.T.F. The concentration of NH_3 in the static chamber rapidly falls, however, due to absorption of gas on the hair of the animals and in any moisture present. Concentrations of NH_3 above 8.5 mgm./l did prove rapidly fatal in the chamber used for gassing. Solis-Cohen and Githens (8) list the fatal dose at 0.2 per cent of inspired air but do not mention if per cent means w/v or v/v nor the species of animal or animals used nor the time of exposure; if v/v is intended, then the concentration corresponds to 1.5 mgm. per l. Exposure to concentrated ammonia fumes is well known to produce violent irritation of the mucosa of the nose and throat. Severe poisoning has occurred from accidents chiefly involving refrigeration units and in two recent reports, one (9) in an ice cream factory and another (10) in an air raid shelter beneath a brewery, tracheitis, bronchitis and bronchiolitis have been described as among the effects of the gas. Experience with the animals used in the present study has been similar. Sections of trachea and lungs, taken at death, were examined histologically by Prof. G. H. Ettinger of the Department of Physiology of Queen's University and he reported extensive congestion and edema with desquamation, leucocytic infiltration and necrosis of the mucosa of the trachea, bronchi and bronchioles. It may be concluded, therefore, that the gassing severely damaged the ciliated mucosa of the respiratory tract in the animals used. Eighteen rabbits and 12 cats served as controls, not exposed to NH_3 but otherwise treated as the gassed animals.

The mean output of R.T.F. with the animals lying prone upon their bellies on the operating table was as follows:

Control rabbits, no NH_3	2.2 ml./kilo/24 hrs.
Ammonia-gassed rabbits.....	3.2 ml./kilo/24 hrs.
Control cats, no NH_3	3.3 ml./kilo/24 hrs.
Ammonia-gassed cats.....	1.9 ml./kilo/24 hrs.

These figures indicate that in spite of the inhalation of NH_3 , the animals continued to excrete about the same amount of R.T.F. There are two possible explanations, first the cilia had not been completely destroyed by NH_3 or secondly that mechanisms other than ciliary drainage accounted for the continued output of R.T.F. The first explanation does not seem likely because of the extensive damage to the mucosa found histologically. Further, in the experiments on the inhalation of live steam reported below, figures similar to the above were obtained and in the steamed animals the tracheal mucosa at death was often necrotic and so rotten that in some animals the cannula sloughed out of the trachea. It seems reasonable to conclude that a mechanism or mechanisms other than ciliary drainage served to excrete amounts of R.T.F. about those of the normal output. On the other hand, it cannot be concluded that non-ciliary mechanisms of drainage can accommodate volumes of R.T.F. greater than the normal output. In fact, from the experiments described below it would seem that these non-ciliary mechanisms are limited in their capacity to excrete only

amounts of R.T.F. about the normal output. Cough is not included in the non-ciliary mechanisms just described because these animals could not cough. The non-ciliary mechanisms involved, therefore, are movements of the lungs due to contraction and relaxation of the muscles associated with the mechanics of respiration and the contractions of the bronchial muscles of Reisseissen.

That drainage of R.T.F. was not efficient in ammonia-treated rabbits and cats was immediately apparent when the animals were arranged for postural pulmonary drainage by tilting the operating table with the animals' heads held downward. Two angles of drainage were selected, 30° and 50° with the horizontal. The animals were placed either at an angle of 0° (prone), 30° or 50° for 2 hours, then shifted to another angle for 2 hours, then to another and so on, the sequence of shifting being made such that a random sampling was obtained.

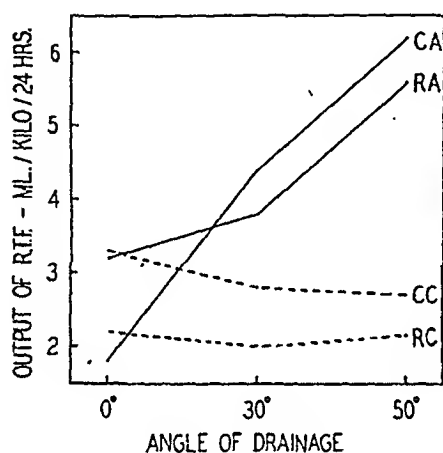


Fig. 1

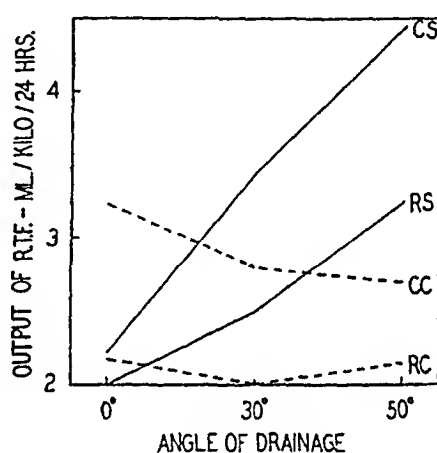


Fig. 2

Fig. 1. The effect of postural pulmonary drainage upon the output of R.T.F. in ammonia-gassed rabbits and cats. CA: cats, ammonia-gassed; RA: rabbits, ammonia-gassed; CC: cats, control, no NH_3 ; RC: rabbits, control, no NH_3 .

Fig. 2. The effect of postural pulmonary drainage upon the output of R.T.F. in rabbits and cats subjected to inhalation of live steam. CS: cats, steamed; RS: rabbits, steamed; CC: cats, control, no steam; RC: rabbits, control, no steam.

The mean output of R.T.F. at each angle of pulmonary drainage was then calculated and these means have been plotted in figure 1 for the 4 groups of animals. It may be seen that postural pulmonary drainage had no effect upon the output of R.T.F. in the control animals but increased the output in the ammonia-gassed animals. These experiments indicate that in rabbits and cats:

a. Inhalation of ammonia augments the output of R.T.F. to double or triple the normal production.

b. Inhalation of ammonia damaged the ciliated tracheo-bronchial mucosa.

c. The non-ciliary drainage mechanisms of respiratory movements and contractions of the bronchial muscles could take care of about the normal output of R.T.F. but not the augmented output in ammonia-gassed animals.

Had the cilia been intact and active in the ammonia-gassed animals, could they have efficiently excreted the augmented output of R.T.F.? It seems likely that

they could have done so from the results of experiments to be reported below in which the drainage of R.T.F. was efficient at greatly increased rates of output of R.T.F. and in which the cilia were normal. Hence it would appear that the ciliary drainage mechanism is one of the most important in the excretion of R.T.F. and that drainage by action of the respiratory movements and contractions of the bronchial muscles can accommodate amounts of R.T.F. of only about the normal output.

The inhalation of steam. Seven rabbits and 3 cats were allowed to inhale live steam directly into the trachea and then arranged for collection of R.T.F. The animals were urethanized, the tracheal cannula inserted and then a stream of live steam added to the inhaled air from a steam kettle on an electric hot plate for a period of from 2 to 45 minutes, the inhaled live steam passing directly into the trachea. A number of other animals were used in addition to those mentioned but their survival was too short to render inclusion of data obtained upon them. At death, sections of the trachea and lungs were examined histologically by Professor Ettinger who reported extensive venous congestion and edema of the trachea with leucocytic infiltration, necrosis and sloughing of the mucosa. In the bronchi and bronchioles the pathological reaction was less severe and in a few sections normal. The damage to the trachea was quite apparent macroscopically.

The mean output of R.T.F. in the steamed animals lying prone upon their bellies was:

Control rabbits, no steam.....	2.2 ml./kilo/24 hrs.
Steamed rabbits.....	2.0 ml./kilo/24 hrs.
Control cats, no steam.....	3.3 ml./kilo/24 hrs.
Steamed cats.....	2.3 ml./kilo/24 hrs.

The control animals were the same as those used for the ammonia-gassed rabbits and cats as all experiments with NH_3 and steam were done at about the same time. These results are similar to those with the ammonia-gassed animals. The conclusions are thus also the same with respect to the relative functions of the different mechanisms responsible for drainage of R.T.F.

The steamed animals were then set up for postural pulmonary drainage at the same three angles as the ammonia-gassed animals. The mean output of R.T.F. at 0° , 30° and 50° angles of drainage has been plotted in figure 2. It may be seen that the results, and hence the conclusions, are similar to those with ammonia-gassed animals.

The capacity for excretion of R.T.F. The above experiments demonstrated that in the absence of normally functioning cilia, the non-ciliary mechanisms of drainage of R.T.F., exclusive of cough and reabsorption, were capable of excreting R.T.F. at the maximal rate of some 2 ml./kilo/24 hrs. in rabbits and cats. In the following experiments it will be shown that with an intact tracheo-bronchial mucosa, the respiratory system has a capacity to excrete many times this amount of R.T.F.

First, an effort was made to augment the output of R.T.F. in rabbits by giving

various cholinergic drugs and measuring the output of R.T.F. with animals arranged consecutively for postural drainage at the angles previously used. Seventy-six experiments were performed upon 21 rabbits using the following doses per kilo body weight given intraperitoneally: 0.005 to 0.05 mgm. carbachol, B.P. (Doryl-Merck), 0.05 to 0.1 mgm. physostigmine salicylate, 0.2 mgm. arecoline hydrobromide, 0.005 to 0.05 mgm. carbaminoyl betamethylcholine chloride (Urecholine-Merck), 0.02 to 0.2 mgm. prostigmine methylsulphate, 1 to 10 mgm. acetyl betamethylcholine chloride (Methohyl-Merck.) None of these drugs had a marked effect upon the volume output of R.T.F. in the doses used and the average output was increased only about 50 per cent. The mean output at the different angles of postural pulmonary drainage was:

At angle 0°.....	3.2 ml./kilo/24 hrs.
At angle 30°.....	3.5 ml./kilo/24 hrs.
At angle 50°.....	3.6 ml./kilo/24 hrs.

Postural pulmonary drainage did not materially affect the output of R.T.F. over that in the prone, 0°, position and hence it may be concluded that drainage at this rate of output is efficient and effective. There was no evidence of damage to the tracheo-bronchial mucosa in these animals, of course.

Cholinergic drugs having proven unsatisfactory in the rabbit, a series of some 40 experiments was performed upon 10 rabbits in which the cervical vagus nerve was stimulated faradically and at intervals after the technique used by Perry and Boyd (5). The rabbits so treated were again arranged in a random manner for collection of R.T.F. at the angles 0°, 30° and 50°. The mean output of R.T.F. was:

At angle 0°.....	7.1 ml./kilo/24 hrs.
At angle 30°.....	7.6 ml./kilo/24 hrs.
At angle 50°.....	7.0 ml./kilo/24 hrs.

The output of R.T.F. in these experiments was over three times the normal, there was no evidence of damage to the tracheo-bronchial mucosa and the output of R.T.F. was not increased by postural pulmonary drainage. Hence it may be concluded that the mechanism of drainage of R.T.F. is geared to handle loads three times the normal—with the ciliated epithelium intact.

A confirmatory experiment was performed upon 10 cats given intraperitoneal injections of pilocarpine nitrate, 0.5 mgm. per kilo body weight. The mean output of R.T.F. in 37 comparisons at different angles of postural pulmonary drainage was:

At angle 0°.....	6.8 ml./kilo/24 hrs.
At angle 30°.....	6.5 ml./kilo/24 hrs.
At angle 50°.....	7.3 ml./kilo/24 hrs.

The results and conclusions deduced therefrom are similar to those with vagus-stimulated rabbits.

There was also available for study a number of sick rabbits, cats and dogs with various types of pathological lesions of the lung and from which greatly increased

amounts of R.T.F. were obtained. From the point of view of the present investigation, interest lay only in the condition of the trachea and bronchi. In one group of some 28 rabbits, about three quarters of the animals exhibited congestion and edema of the tracheal mucosa. In these rabbits the output of R.T.F. was:

At angle 0°.....	1.7 ml./kilo/24 hrs.
At angle 30°.....	32.7 ml./kilo/24 hrs.
At angle 50°.....	34.1 ml./kilo/24 hrs.

It is obvious that in these rabbits the loss of normal ciliary function materially reduced the capacity of the lung to excrete the heavy load of R.T.F.

On the other hand, in a group of 44 cats with congestion, edema, leucocytic infiltration and other damage to the lung alveoli but no appreciable damage to the mucosa of the trachea, bronchi and bronchioles, the mean output of R.T.F. was:

At angle 0°.....	15.0 ml./kilo/24 hrs.
At angle 30°.....	8.5 ml./kilo/24 hrs.
At angle 50°.....	16.1 ml./kilo/24 hrs.

These experiments attest the capacity of the cat respiratory tract to cope with an output of R.T.F. some 8 times the normal—with the ciliated mucosa intact. There were individual examples where the output of R.T.F. in these cats rose to 50 to 100 times the normal rate and postural pulmonary drainage still did not affect the output of R.T.F. It seems quite evident that the mechanism for excreting R.T.F. in cats is geared to handle many times the normal output.

In a group of 7 dogs with lesions of the alveoli similar to those seen in the cats but also with histologically normal tracheo-bronchial mucosa, there was an augmented output of R.T.F. but no effect due to postural pulmonary drainage upon the rate of output. The normal output of R.T.F. in adult dogs of 15 to 40 lbs. body weight averages about 0.5 ml./kilo/24 hrs. and the output is not increased by postural pulmonary drainage. In the 7 dogs noted above, the mean output of R.T.F. was:

At angle 0°.....	8.7 ml./kilo/24 hrs.
At angle 30°.....	6.8 ml./kilo/24 hrs.
At angle 50°.....	6.5 ml./kilo/24 hrs.

These experiments demonstrate also that the dog is capable of excreting 15 times or so the average normal output of R.T.F. and the tracheo-bronchial mucosa was normal.

CONCLUSIONS

The factors responsible for excretion of R.T.F. up from the lungs and through the trachea in urethanized, tracheal-cannulated rabbits, cats and dogs have been listed as 1, ciliary drainage; 2, the movements due to action of the voluntary muscles of respiration, and 3, the contractions of the bronchial muscles of Reis-

seissen. Cough and reabsorption also play a part but not in the above particular experimental animals.

If the drainage mechanisms are ineffective or incapable of excreting R.T.F. as fast as it is produced, then an increased output of R.T.F. should result from postural pulmonary drainage.

In the normal, urethanized rabbit, cat and dog, postural pulmonary drainage does not increase the output of R.T.F. and hence the mechanisms for drainage must be sufficient and effective.

When the ciliated mucosa lining the trachea of rabbits and cats is damaged by the inhalation of ammonia or live steam, the output of R.T.F. is doubled or tripled when the animals are posturally drained but drainage is incomplete and at about the normal rate when the animals are not posturally drained. Hence mechanisms 2 and 3 above may be concluded as geared to handle not more than the normal load of R.T.F.

When the output of R.T.F. in rabbits and cats is doubled or tripled by giving cholinergic drugs or by faradic stimulation of the cervical vagus nerve, the output is not augmented by postural pulmonary drainage, the ciliated mucosa is undamaged and hence it may be concluded that the ciliary drainage mechanism is geared to handle loads of R.T.F. greater than the normal.

In sick cats and dogs with a normal tracheo-bronchial mucosa but with a congestion of the pulmonary alveoli, postural drainage did not augment outputs of R.T.F., which, on the average, were 8 to 15 times the normal and in individual animals went as high as 50 to 100 times. Hence the reserve capacity of the cilia to excrete R.T.F. must be very great.

Cough is not likely to be useful and productive, therefore, except if there be extensive damage to the tracheo-bronchial mucosa or where the cilia are bathed in a markedly non-physiological medium, such as pus. Postural pulmonary drainage is likely to be effective only under the same conditions.

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CARBOHYDRATE REGULATION UNDER SEVERE ANOXIC CONDITIONS¹

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Most of the literature indicates that anoxia causes hyperglycemia (5, 6, 12, 13, 14, 16, 18, 19), although some investigators report no change (1, 8) or even hypoglycemia (15, 21) under some conditions. The confused state of the evidence is well summarized in the monograph by Van Liere (22).

Sundstroem and Michaels (21), using rats, showed that under anoxic conditions (260 mm. Hg) maintained for two weeks there is at first a slight hypoglycemia, followed by several days of hyperglycemia, and terminated with hypoglycemia. Gellhorn and Packer (9) found differences in the effects of anoxia on rabbits which were apparently referable to exposure times: short periods acted antagonistically to insulin hypoglycemia, while longer periods of two hours aggravated hypoglycemia.

Lewis *et al.* (15) found in rats a slight increase in blood sugar during anoxia stimulated by atmospheres containing more than 7 per cent oxygen, while lower oxygen levels produced hypoglycemia. As early as 1914, Cannon (3) suggested that asphyxia stimulates production of the adrenal medullary hormone, and might be expected to evoke an increase in blood sugar. Schulze (20) and Feldman *et al.* (6) have since suggested that anoxic hyperglycemia may be due to stimulation of the adrenal glands. In this connection Emerson and Van Liere (4) have shown that anoxia causes depletion of adrenalin content of these organs.

In the present experiments the effects on carbohydrate levels of oxygen lack, of attendant manipulation and other exciting factors were examined. The changes in blood sugar on rapid evacuation of the air chamber to rather extreme conditions (260–160 mm. Hg) followed by rapid return to sea-level conditions, the incidence of convulsions, and problems of adrenal involvement in anoxia were considered in several experimental series. The effects on storage of carbohydrates were also investigated.

METHODS. Room and chamber temperatures were kept between 25°C. and 28°C. Gas pressures were reduced rapidly, within 2 minutes, to the stated values. All heights were derived by comparison with the altitude-pressure table given by Armstrong (2). Male rats were used throughout.

Glass barometric chambers, each with a capacity of 9 liters, were arranged for testing individual rats, or in a few cases two or three small rats. At S.T.P., each was ventilated at the rate of 2 liters of fresh air per minute, but this rate was increased in inverse proportion to the decreased pressure within the chamber. Thus, when the pressure had been reduced to 380 mm., the ventilation rate in

¹ Grateful acknowledgment is made of aid received in this investigation from the Endocrinology Fund of the National Research Council.

liters per minute was more than double the 2 liters per minute measured at S.T.P. Pressure was kept constant to within ± 5 mm. by a specially-designed apparatus in which evacuation was somewhat excessive, the excess being relieved by atmospheric air bubbling through a column of mercury of fixed depth. Periodic analysis showed that the partial pressure of CO_2 never exceeded 1.5 mm. Hg or 0.6 per cent by weight, at 27,000 ft. simulated atmosphere, even when large rats were used.

Blood-sugar determinations were made according to the micro-method of Folin and Malmros (7), samples being taken from the tail of each rat. The progressive changes in blood sugar which occurred under many different anoxic conditions have been observed. The effects of repeated manipulation of rats were determined, both with and without exposure to low oxygen tensions. In some groups, liver, muscle and heart glycogen levels were observed.

The acclimatized rats used in these experiments had been exposed to 27,000 ft. altitude equivalent for several 4-hour periods during the month preceding their use. As a test for acclimatization the group (10 animals) was exposed to 162 mm. Hg (37,000 ft.) for one hour. Ordinarily, all unacclimatized animals died during such exposure tests.

Adrenalectomized animals, and the effects of adrenalin (0.02 mgm./100 gram intraperitoneally), were tested in relation to anoxia. The adrenalectomized rats were maintained on 0.2 mgm. desoxycorticosterone acetate per day.

A summary of the various experimental groups in which the effects of anoxia on carbohydrate levels have been studied is given in table 1. In almost all cases, the few exceptions being noted, exposures were made at the simulated altitude of 27,000 ft., or 258 mm. Hg barometric pressure.

RESULTS. Experimental results are given here only in brief, since the accompanying graphs and tables indicate much more clearly the actual blood glucose changes, time factors and other conditions. Over 200 rats were used in the different series tested.

Control animals (groups 1, 2; figs. 1, 2) showed only slight variations in blood sugar, amounting to no more than ± 10 mgm. per cent.

Uninterrupted exposure of fasted animals to 258 mm. barometric pressure (27,000 ft. altitude equivalent) for less than one hour produced a marked rise in blood sugar, but exposure for longer periods up to four hours caused irregular changes, the averages of which are close to normal (group 3, fig. 3).

In some cases, anoxic exposures (to 258 mm. Hg barometric pressure) were interrupted by rapid returns to sea level 6 to 8 times during a 4-hour period (figs. 4, 5, 6, 7, 8). At each such interruption blood samples were taken. These showed blood-sugar changes which varied markedly with the various degrees of fasting imposed. Well-fed animals showed an initial rise in blood sugar followed by return to normal levels (group 4, fig. 4).

Twelve-hour fasted rats demonstrated a similar initial hyperglycemia followed by a steady drop to hypoglycemic levels (60 mgm. per cent; group 5, fig. 4). Return to sea level is followed by a relatively rapid return to normal glycemic values (group 6, fig. 5). If, however, the animals are prefasted for 48 hours, the

same type of exposure produces changes qualitatively similar to those observed in well-fed animals (group 7, fig. 6).

TABLE 1

GROUP	NO. CASES	FIG. NO.	EXPERIMENTAL CONDITIONS
1	10	1	Fasted 12 hours. Repeated handling only, at sea level
2	9	2	Fasted 12 hours. Repeated 5-second exposures to 258 mm. Hg
3	58	3	Fasted 12 hours. Continuous exposures for different time periods
4	6	4	Fully fed; 4-hour total exposure, withdrawals for blood sampling
5	19	4	Fasted 12 hours. Conditions as in group 4
6	6	5	Same as group 5, but recovery period observed for 2 hours
7	5	6	Same as group 4, but rats fasted for 48 hours
8	10	7	Acclimatized rats fasted 12 hours. Conditions as in group 4
5, 7, 9	30		Glycogen levels after 4-hour exposures under different absorptive conditions (table 2)
10	5	8	Adrenalectomized rats, fully fed; repeated handling at sea level
11	7	8	Adrenalectomized rats, fully fed; exposed to 258 mm. with repeated samplings
12	6	9	Adrenalin effect without anoxie exposure
13	15	9	Adrenalin given after 4 hours' anoxia, and exposure continued
14	6	10	Adrenalin effect immediately after 4 hours' anoxia
15	4	10	Adrenalin given 45 minutes after 4 hours' anoxia
16	21	11	Blood glucose levels during rapidly induced anoxie convulsions

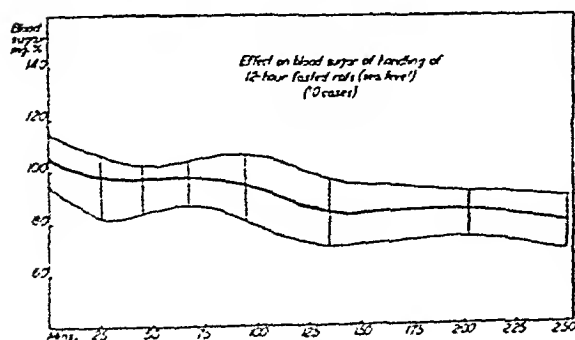


Fig. 1

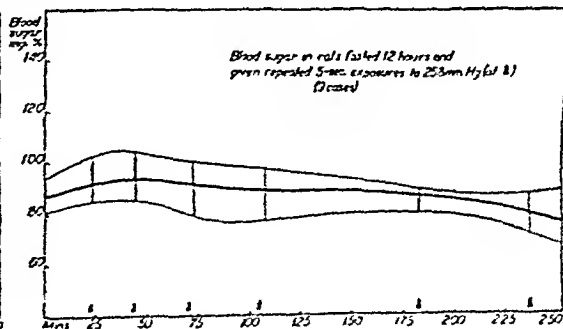


Fig. 2

Figs. 1 and 2. Control experiments showing course of blood sugar under conditions indicated on graphs. (In graphs like these, 1 and 2, the area within the light lines represents the range of the experimental values and the dark line the average course.)

Acclimatized rats which were prefasted for 12 hours before the anoxic exposure showed no reduction in blood-sugar level, and hence were similar in the latter respect to the well-fed unacclimatized animals (group 8, fig. 7).

No marked changes were observed in the liver or muscle glycogen values of either well-fed, 12-hour or 48-hour prefasted animals subjected to this experiment. Heart glycogen, however, suffered reduction in all cases (groups 5, 7, 9; table 2).

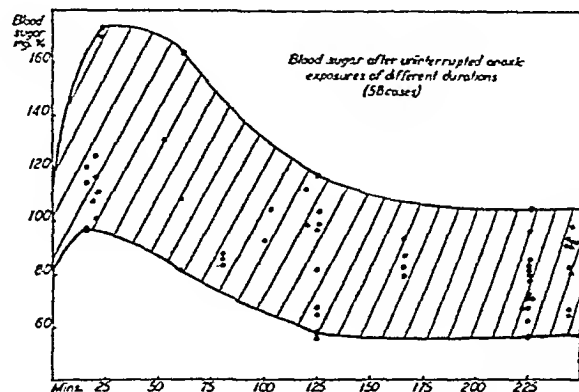


Fig. 3. Blood sugar changes in 12-hour fasted rats exposed to anoxia (258 mm. Hg) for different time periods.

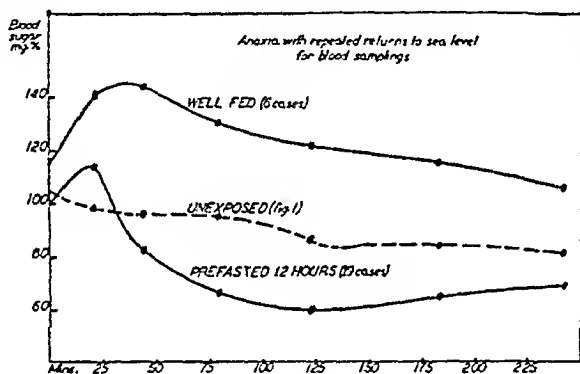


Fig. 4

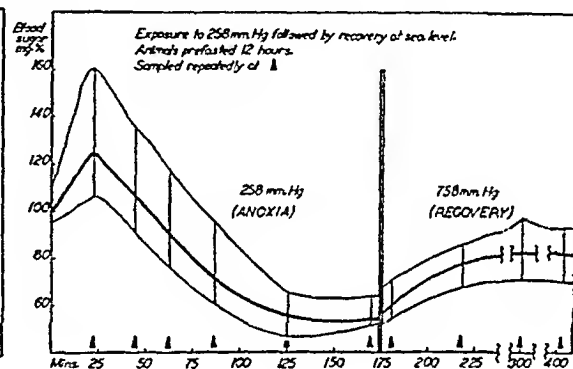


Fig. 5

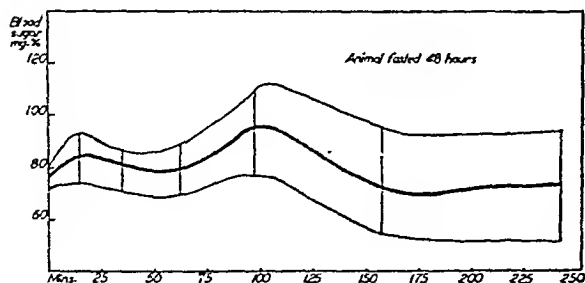


Fig. 6

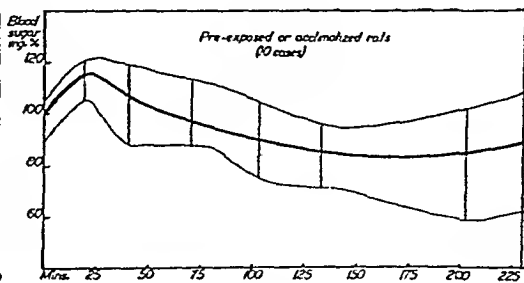


Fig. 7

Figs. 4, 5, 6, 7. Blood glucose changes in rats exposed to anoxia; repeated blood samplings during experimental period. See data on respective figures.

Well-fed adrenalectomized rats exposed to 258 mm. Hg barometric pressure for 4 hours, with 7 periodic returns to sea level, demonstrated a continuous trend toward hypoglycemia (group 11, fig. 8).

The usual effect of adrenalin on the blood sugar (group 12, fig. 9) is annulled

TABLE 2
Glycogen levels after anoxia
(27,000 ft. for 4 hrs.)

	TIME OF FAST		
	4 hrs.	16 hrs.	50 hrs.
<i>Anoxic rats (each number is mean of 6 determinations)</i>			
Liver.....	1.13	0.45	0.26
Muscle.....	0.59	0.43	0.37
Heart.....	0.40	0.49	0.38
<i>Unexposed controls (each number is mean of 4 determinations)</i>			
Liver.....	1.22	0.45	0.26
Muscle.....	0.56	0.47	0.30
Heart.....	0.49	0.65	0.58

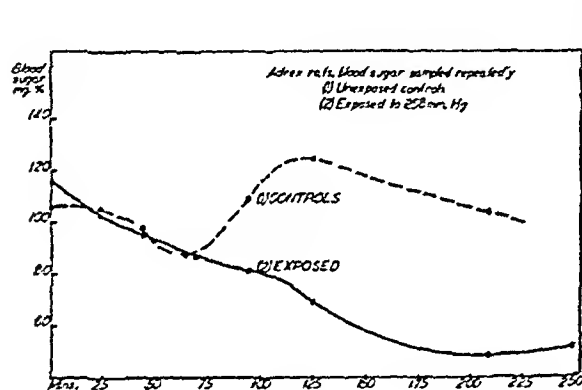


Fig. 8

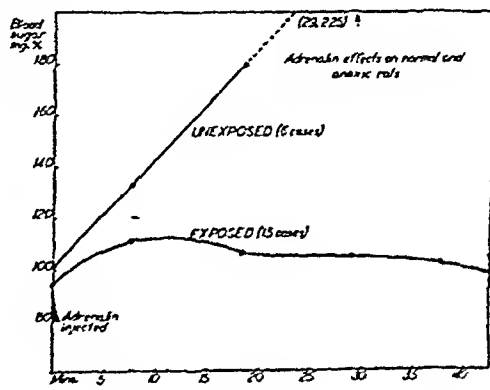


Fig. 9

Fig. 8. Course of blood sugar in adrenalectomized rats treated as indicated on graph.

Fig. 9. Effects of adrenalin on rats during anoxic exposure, compared to non-anoxic cases.

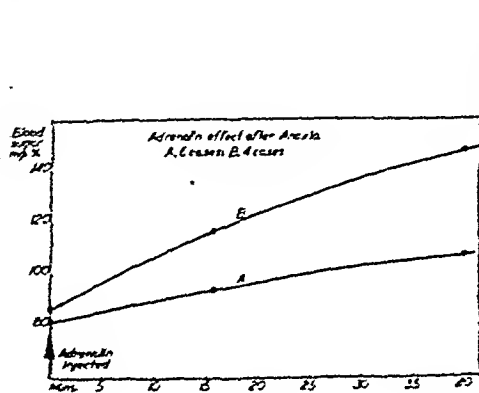


Fig. 10

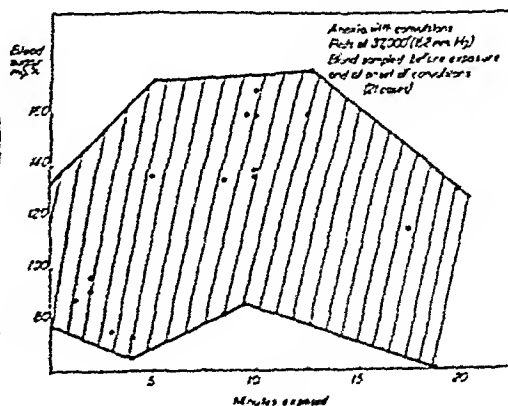


Fig. 11

Fig. 10. Adrenalin administered to rats after a four-hour exposure to anoxia (27,000 ft.). (A) Effect of adrenalin immediately after anoxia and return to sea level. (B) Adrenalin injected after 45 minutes' recovery at sea level following anoxia.

by pre-exposure of the animals for 4 hours at 258 mm. barometric pressure (group 13, fig. 9). Recovery of the normal adrenalin action requires over 45 minutes at sea level (group 14, 15; fig. 10).

The blood-sugar may be normal or high at the initial stages of anoxic convulsions produced by rapid exposure to a barometric pressure of 162 mm. Hg (group 16, fig. 11). In addition, about 20 per cent of the fasted animals exposed as in figures 4 and 5 died in convulsions after 2 to 3 hours of exposure. The blood sugar of these rats was following the same course as others in the group up to the time of withdrawal, being between 60 to 70 mgm. per cent at the 120 to 130 minute stage.

DISCUSSION. It is apparent that anoxia may produce complex physiological effects, and many of these are reflected by the blood-sugar changes. At least two considerations are of distinct interest, namely, *a*, the specific or direct chemical effects produced in the tissues by insufficient oxygenation, and *b*, the compensatory reactions which are set in operation by the anoxic disturbances. Moreover, an animal which shows emotional and physical excitement because of the rapid reduction in barometric pressure and the consequent respiratory difficulties may show internal bodily changes which are additional to the foregoing.

The discrepant accounts in the literature of changes found in the blood-sugar level of anoxic animals are not surprising in view of the results reported here. Two types of exposure—continuous and discontinuous—were considered, and two different results have been obtained: in one (fig. 3), no marked change in blood sugar was produced after the hyperglycemia of the first 30 to 60 minutes; in the other (fig. 4), a definite and rather severe reduction was observed. The degree of fasting experienced by the animal is important in this latter respect. Rats exposed to anoxic conditions showed *a*, no marked changes when well fed (fig. 4); *b*, hypoglycemia if moderately fasted (figs. 4, 5), or *c*, no marked change after a prolonged fast (fig. 6). Acclimatization also inhibits anoxic blood-sugar changes (fig. 7).

The initial rise in blood sugar within the first 30 to 60 minutes of exposure may possibly be due to augmented activity of the adrenal mechanism, as recognized by previous investigators (3, 4, 6, 20). It was reported by Kellaway (13), however, that the initial sugar rise was not entirely accounted for by excitement of the animal, since it was obtained in anesthetized animals. Gellhorn and Packer (10) suggested that the blood-sugar level might be more sensitive to adrenalin during the preliminary phases of anoxia. Thus, the initial rise might be due to more than one factor, both emotional and chemical effects possibly contributing towards it.

Much of the evidence reported herein emphasizes further the importance of the adrenal mechanism in body regulations under low barometric pressures. Bilaterally adrenalectomized rats maintained on D.C.A. showed an initial fall in blood sugar, instead of the rise found in normal animals. This result has also been established by previous investigators (6, 18) under slightly different experimental methods. Carbohydrate reserves or the mobilization forces are

apparently inadequate in the absence of the adrenal tissues. The possibilities of gluconeogenesis under anoxic conditions have not been considered in the present work.

There is no marked difference in the curve of blood sugar vs. time, shown by experimental rats which are simply handled for blood sampling purposes, compared to those which are placed in a chamber which is rapidly evacuated to 258 mm. and immediately returned to 760 mm., and the rats then handled and sampled (figs. 1, 2).

The gradual but continuous reduction in blood-sugar level which is shown in figures 4 and 5 (when compared with fig. 3) appears to be due to anoxia, in addition to either repeated excitement or repeated return to sea-level conditions, or both the latter factors. The usual reserves of glycogen evidently remain unchanged, as shown by the figures in table 2, as well as by the relatively rapid recovery to normal (fig. 5).

The mechanism for releasing reserves by sympathico-adrenal glycogenolysis is suppressed during anoxia, or at least greatly inhibited. Glycogenolytic mechanisms are interfered with by the severe anoxia, and evidently cannot maintain the normal blood-sugar level even though carbohydrates held in reserve appear to be adequate. Under low oxygen tensions, reduced effectiveness of adrenalin is probably an important factor, but undoubtedly other considerations are involved. In this connection it may be observed that Feldman *et al.* (6) and McQuarrie *et al.* (17) report that pancreatic production of insulin, as well as the normal effectiveness of this hormone, may continue even under severe anoxia.

Well-fed animals do not suffer from hypoglycemia such as that exhibited by mildly-fasted subjects. On the other hand, the liberation of blood sugar from the glycogen depots would appear to require an easily accessible supply of oxygen, for even these well-fed animals do not respond to adrenalin to the normal extent after exposure to anoxia. These results agree with those of Gellhorn and Packer (10).

The blood-sugar levels of adrenalectomized and fasted unoperated animals are similar after the first 100 minutes of exposure to 27,000 ft. altitude equivalent (figs. 4, 5, 8). Evidently the adrenal glands and/or their hormones are rendered nearly ineffective by severe anoxia. Giragossintz and Sundstroem (11) have suggested that severe anoxia presents some aspects of cortico-adrenal insufficiency. It appears as though absorption of glucose may be associated with adrenal activity, since the adrenalectomized animals that were exposed were well fed up to the time of exposure, yet they suffered from hypoglycemia. This is unlike well-fed normal animals, which usually did not exhibit markedly sub-normal blood-glucose levels. This subject is under investigation.

The fact that animals may be protected against anoxic hypoglycemia by feeding carbohydrates does not imply that they are protected against anoxia, *per se*, especially since blood-sugar levels and anoxic convulsions have no very close correlation (fig. 11). It may be that both severe anoxia and profound hypoglycemia would produce somewhat similar disturbances of normal life processes. Both conditions may result in insufficient usage of carbohydrates. Accordingly,

overdosage of insulin, without regard to the oxygen tension, would produce convulsions followed by death. Thus, inadequate oxidation or utilization of carbohydrates may be the crux of both problems.

SUMMARY

Rapidly-induced, severe anoxia produces in rats a rise in blood sugar lasting for periods up to one hour. This is probably due to excitation of the sympathico-adrenal mechanism. The occurrence of hypoglycemia in adrenalectomized animals tested under the same conditions supports this hypothesis.

✓ Rats fasted for short periods up to 12 hours and then exposed to an anoxic atmosphere simulating 27,000 ft. altitude (258 mm. Hg) undergo severe hypoglycemia, although the carbohydrate stores remain adequate.

The normal hyperglycemic action of adrenalin is not observed in rats which are injected after exposure to 258 mm. Hg for 4 hours, and the anoxia then continued. No significant improvement of the adrenalin-resistant condition is observed until about 45 minutes after an animal has been removed from the low-pressure chamber. These facts shed some light on the hypoglycemic condition which eventually develops in normal animals exposed to anoxia as described.

Well-fed normal rats do not show the usual hypoglycemic reaction observed in moderately-fasted animals exposed to 258 mm. Hg barometric pressure. Likewise, anoxia-acclimatized rats exhibited no severe changes in blood-sugar level during these anoxic exposures.

The ultimate blood sugar values in well-fed adrenalectomized rats exposed to anoxia are lower than those of fasted unoperated individuals. This is further evidence that in normal animals the adrenal glands or their hormones are at least partially incapacitated by severe anoxia.

✓ Rapid reduction of barometric pressure followed by rapid return to sea level, the whole exposure covering not more than 20 seconds, results in only slight changes in the blood-glucose levels of moderately-fasted rats.

Heart glycogen is significantly reduced by 4 hours' anoxia at 258 mm. Hg barometric pressure. Under similar conditions liver and muscle glycogen levels, however, are found within normal limits. This suggests that anoxia may produce considerable strain on cardiac function.

Blood-sugar levels may either rise, fall, or remain unchanged under anoxia (258 mm. Hg barometric pressure), according to experimental conditions. Effects apparently depend on the absorptive condition of the animal, length and degree of anoxic exposure, and also amount of pre-exposure or acclimatization. This may explain apparent discrepancies in the literature regarding the effect of anoxia on blood-sugar level. ✓

It may be stated that in general all normal, unoperated rats show hyperglycemia during the first 60 to 90 minutes of exposure to barometric pressures (ca. 260 mm. Hg), followed by moderate hypoglycemia during the next 2 to 4 hours. The late hypoglycemic phase does not appear, however, in well-fed or acclimatized rats. Adrenalectomized rats show progressive and eventually severe hypoglycemic responses when exposed similarly to anoxic conditions.

While well-fed animals do not experience hypoglycemia on exposure to anoxic conditions, they are frequently not protected against convulsive seizures. Anoxic convulsions may indeed occur at any blood-sugar level within wide limits.

Anoxic and hypoglycemic convulsions may be essentially similar fundamentally; possibly they are referable to reduced oxidation or utilization of carbohydrate materials.

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THE EFFECT OF SULFONAMIDES ON THE BLOOD OXYGEN AND CARBON DIOXIDE CAPACITY, ARTERIAL SATURATION, AND BLOOD PIGMENTS

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Since the introduction of the sulfonamide drugs as effective agents in the treatment of bacterial infection, a few undesirable reactions resulting from their use have been observed and are undergoing investigation. Among these is the production of non-oxygen carrying blood pigments such as sulfhemoglobin or methemoglobin. Numerous reports may be found in the literature giving evidence that the above pigments are formed in the blood of humans and animals by administration of sulfanilamide with, or without, accompanying cyanosis. However, in regard to other sulfonamides, little is available either regarding their possible production of sulf- or methemoglobin or their effects upon the oxygen and carbon dioxide combining capacity of the blood.

Chesley (2) in eight patients showing cyanosis during sulfanilamide therapy ruled out the possibility of sulf- or methemoglobinemia since measurements of the blood carbon monoxide capacity indicated all the hemoglobin to be in the active form. Harris and Michel (5), however, in a study of 476 patients, found methemoglobin present roughly in proportion to the amount of blood sulfanilamide. Hartman, Perley and Barnett (6), using a spectroscopic method for the determination of methemoglobin reported they were able to demonstrate the presence of methemoglobin in every cyanotic case receiving 0.1 gram/kgm. or more of the drug per 24 hours, with marked individual variations however both as regards rate and degree to which methemoglobin accumulates. Vigness, Watson and Spink (11) found methemoglobin in the blood of sulfanilamide-treated patients but in quantities believed too small to contribute appreciably to cyanosis. Webb and Kniazuk (12), employing a sensitive spectrophotometric method, reported that in rats receiving large daily doses of sulfanilamide a variety of effects were observed: some animals' blood contained either met- or sulfhemoglobin; other bloods were normal; while in some animals an unidentified foreign pigment was observed. Wendel, Wendel and Cox (13), from combined spectrophotometric and gasometric analyses of blood in nine cyanotic subjects, reported the abnormal color to be due for the most part to methemoglobin.

Observations of the effect of the sulfonamides upon the oxygen-carrying capacity or arterial saturation of the blood are few. King and Leslie (8) report in eight cases cyanosed after sulfanilamide therapy that a lowered oxygen content of arterial blood was not a significant factor. Mull and Smith (10), however, observed a sharp fall in oxygen content and capacity of the blood following treatment of a patient with sulfanilamide.

The purpose of the present work was to study, primarily, the effect of single

therapeutic doses of various sulfonamides (sulfanilamide, sodium sulfapyridine, sulfathiazole, sodium sulfadiazine, and sulfaguanidine) upon the oxygen capacity and arterial saturation of the blood and, secondarily, the effect of such doses upon the blood pigments and alkaline reserve.

The experimental evidence in regard to the effect of the sulfonamides upon acid-base balance is conflicting. Marshall, Cutting and Emerson (9) have reported that sulfanilamide produces an acidosis in dogs, while more recently Free, Davis and Myers (4) have found that therapeutic quantities do not result in any marked change in the acid-base balance.

METHODS. Forty-one experiments upon eight normal dogs were completed. All experiments were performed upon animals which had been fasted for at least 12 to 16 hours. The procedure followed consisted in first obtaining, by puncture of the femoral artery, a blood sample for control analyses of oxygen capacity, carbon dioxide combining capacity, arterial percentage saturation, and blood pigments. Single therapeutic doses (0.1 gram/kgm.) of the various sulfonamides were then administered orally and blood samples taken at one-half or one-hour intervals for the following five or six hours for determination of the blood sulfonamide concentration and blood pigments. At the peak of the drug's absorption, as measured by the blood concentration, a second similar arterial blood sample was obtained and its oxygen capacity, carbon dioxide capacity, concentration of blood pigment, and in some experiments, the percentage arterial saturation determined.

Oxygen capacity and content were determined on the Van Slyke manometric apparatus according to the method of Van Slyke and O'Neill. Carbon dioxide combining capacity or alkaline reserve (T_{40}) defined as "the CO_2 content (in volumes per cent) of oxygenated blood equilibrated at 37°C . at a pressure of 40 mm. carbon dioxide" was determined by a method recommended by Horvath, Consolazio and Dill (7). Oxygen capacity, defined as "the oxygen content (in volumes per cent) in oxygenated blood equilibrated at 37°C . at approximately 40 mm. carbon dioxide pressure and at 180 to 190 mm. oxygen pressure for fifteen minutes" was also determined by the method recommended by Dill (7). Sulfonamides were determined by the micro photolorimetric method of Britton and Marshall (1) and blood pigments (methemoglobin, sulfhemoglobin, and total hemoglobin) by the method of Evelyn and Malloy (3).

EXPERIMENTAL. The use of the above procedures and methods permitted a comparison to be made between normal or control values of oxygen and carbon dioxide capacity, percentage arterial saturation and blood pigments and experimental values obtained when the concentration of the various sulfonamides in the blood was highest.

In tables 1 through 5 the effects of the respective sulfonamides upon the oxygen and carbon dioxide capacity, arterial saturation, and the percentage changes resulting in each experiment are presented. Mean percentage changes in oxygen capacity ranged from -4.1 volumes per cent with sodium sulfadiazine to -9.5 volumes per cent with sulfaguanidine. Blood concentrations existing at the time of the second arterial blood sample are also reported and represent

the highest concentration attained during the experiment. Four experiments were conducted to determine the normal variation in oxygen capacity of dogs' blood under conditions similar to those existing during the drug experiments. These results are indicated in table 6. Physiological variation in oxygen capacity in the normal dog during the course of a day probably does not exceed (\pm) 2-3 per cent; the greater changes determined (\pm) 4.8 per cent are doubtless

TABLE 1

Effect of sulfanilamide on oxygen and carbon dioxide capacity of blood (T_{40})

DATE	SUBJECT	CONTROLS		BLOOD CONC.	EXPERIMENTAL			CHANGES	
		O ₂	T ₄₀		O ₂	T ₄₀	Saturation	O ₂	T ₄₀
		vols. per cent	vols. per cent				per cent	per cent	per cent
2/28/42	Dog D	14.1	43.3	6.6	13.9	44.9	99.6	-1.4	+3.6
4/ 2/42	Dog D	18.1		8.5	16.4			-9.4	
6/ 3/42	Dog J	13.5	41.5	8.8	12.7	43.6		-5.9	+4.8
6/ 6/42	Dog D	18.6		14.1	15.1	41.0		-18.8	
6/10/42	Dog G	19.6	40.0	12.0	19.6	43.5		0.0	+8.0
6/10/42	Dog I	14.8	40.9	10.7	15.1	41.9		+2.0	+2.4
6/17/42	Dog J	13.9	41.4	9.0	15.6	43.0		+10.9	+3.7
6/17/42	Dog G	20.4	38.5	13.3	18.0	41.0		-11.7	+6.1
Mean.....								-4.3	+4.8

TABLE 2

Effect of sodium sulfapyridine on oxygen and carbon dioxide capacity of blood

DATE	SUBJECT	CONTROLS		BLOOD CONC.	EXPERIMENTAL		CHANGES	
		O ₂	T ₄₀		O ₂	T ₄₀	O ₂	T ₄₀
		vols. per cent	vols. per cent	mgm. per cent			per cent	per cent
7/29/41	Dog B	22.2		7.7	20.8		-6.3	
8/ 1/41	Dog A	23.2		6.5	20.0		-13.8	
8/19/41	Dog B	26.7	42.6	9.8	23.5	38.6	-12.0	-9.4
8/22/41	Dog A	19.8	41.0	8.3	17.6	37.0	-11.1	-9.7
9/ 9/41	Dog B	23.5	36.4	9.8	21.9	39.2	-6.8	+7.1
9/19/41	Dog B	20.5	42.5	7.7	19.4	38.0	-5.4	-10.5
10/30/41	Dog B	23.1	40.4	6.8	22.3		-3.5	
Mean.....							-8.4	-5.6

due to errors involved in gas analysis. Mean percentage changes in the carbon dioxide capacity (T_{40}) varied from a -5.6 volumes per cent for sodium sulfapyridine to a +4.8 volumes per cent for sulfanilamide and sulfathiazole.

Determinations of the blood pigments (methemoglobin, sulfhemoglobin, and total hemoglobin) made at frequent intervals (one-half to one hour) throughout an experiment failed to reveal amounts which could be considered of physiological significance. The amounts of methemoglobin detected were quite small

and did not exceed 3 per cent of the total hemoglobin in any experiments. Sulfhemoglobin was detected only in experiments in which sulfanilamide or

TABLE 3
Effect of sulfathiazole on oxygen and carbon dioxide capacity of blood

DATE	SUBJECT	CONTROLS		BLOOD CONC.	EXPERIMENTAL			CHANGE	
		O ₂	T ₄₃		O ₂	T ₄₃	Saturation	O ₂	T ₄₃
		<i>vols. per cent</i>	<i>vols. per cent</i>	<i>mgm. per cent</i>			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
9/23/41	Dog B	23.0	39	3.5	20.1	41.5		-12.6	+6.0
9/30/41*	Dog B	22.0	38.6	7.7	20.2	45.5		-8.2	+15.1*
10/ 6/41*	Dog B	24.1	38.4	5.2	20.9	46.5		-13.2	+17.4*
12/ 2/41	Dog B	22.2	43.7	9.2	22.0	44.0	93.8	-0.9	+0.7
12/18/41	Dog D	16.9	40.4	6.9	16.7	42.2	95.8	-1.8	+4.3
4/21/42	Dog E	12.3	45.5	7.0	12.3	43.6	91.0	-0.0	-4.2
4/23/42	Dog E	15.5	43.5	4.5	16.2	42.5		+4.4	-2.3
4/27/42	Dog G	18.6	36.6	6.8	18.4	41.0	95.5	-1.1	+10.7
4/14/42*	Dog D	17.3	39.2	2.3	17.1	40.0	96.9	-1.2	+2.0
5/13/42	Dog I	20.4	40.8	6.3	18.5	40.2		-9.1	-1.5
5/25/42	Dog J	15.0		6.0	14.2		97.7	-5.3	
Mean.....								-4.5	+4.8

* Sodium salt of sulfathiazole.

TABLE 4
Effect of sodium sulfadiazine on oxygen and carbon dioxide capacity of blood

DATE	SUBJECT	CONTROLS			BLOOD CONC.	EXPERIMENTAL			CHANGE	
		O ₂	T ₄₃	Satura- tion		O ₂	T ₄₃	Satura- tion	O ₂	T ₄₃
		<i>vols. per cent</i>	<i>vols. per cent</i>	<i>per cent</i>	<i>mgm. per cent</i>			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
10/ 3/41	Dog B	20.0	42.8		5.9	21.4	42.9		+6.5	+2.3
10/17/41	Dog B	21.5	49.0		6.0	18.9	44.8	97.8	-12.1	-8.6
10/24/41	Dog B	21.4	44.5		8.7	19.1	45.0		-10.7	+1.1
3/28/42	Dog F	22.1	43.8	92.9	12.6	22.8	36.0	88.7	+3.0	-17.8
4/17/42	Dog D	18.7	43.5		12.9	16.6	42.5	90.7	-11.2	-2.3
4/17/42	Dog G		42.2		4.6	19.3	42.5	98.8		+0.7
4/29/42	Dog E	11.2	40.0		9.8	10.3	38.2	94.0	-8.0	-4.5
5/ 2/42	Dog D	18.0	39.0		9.9	17.8	40.0		-1.1	+2.5
5/ 5/42	Dog G	20.5	44.2	97.5	7.1	20.5	43.0	97.5	0.0	-2.7
5/18/42	Dog I	18.8	40.0		12.6	19.2	39.0	97.3	+2.1	-2.5
5/21/42	Dog J	17.3	41.0		8.2	14.9	40.6		-13.8	-1.0
Mean.....									-4.1	-3.0

sodium sulfapyridine were administered and the amount of sulfhemoglobin in any experiment did not exceed 2 per cent of the total hemoglobin. Since these quantities of methemoglobin and sulfhemoglobin are only slightly greater than

the limit of sensitivity (0.2 gram per cent and 0.1 gram per cent for methemoglobin and sulfhemoglobin respectively) of the method employed, detailed results of these determinations are not included. The observation that only small quantities of methemoglobin or sulfhemoglobin are produced by single doses of the sulfonamides in the dog is substantiated by the fact that in the majority of experiments only slight mean reductions (less than 10 per cent) in the oxygen capacity were observed.

Percentage arterial saturation was determined in 17 experiments while blood concentrations of the various sulfonamides were at their peak level. Assuming

TABLE 5

Effect of sulfaguanidine on oxygen and carbon dioxide capacity of blood

DATE	SUBJECT	CONTROLS			BLOOD CONC.	EXPERIMENTAL			CHANGE	
		O ₂	T ₄₀	Saturation		O ₂	T ₄₀	Saturation	O ₂	T ₄₀
		vols. per cent	vols. per cent	per cent	mgm. per cent			per cent	per cent	per cent
11/18/41	Dog B	21.1	43.0	94.8	4.0	18.4	41.5	93.6	-12.8	-3.5
4/ 9/42	Dog G	20.1	40.1	94.2	4.0	18.6	40.5	93.5	-7.5	+0.9
5/ 7/42	Dog D	17.3	33.5	98.0	4.1	15.9	31.0	95.5	-8.3	-7.5
Mean.....									-9.5	-3.4

TABLE 6

Normal variations in oxygen capacity of blood

DATE	SUBJECT	OXYGEN CAPACITY		CHANGE
		Control	5-6 hr. sample	
		vols. per cent	vols. per cent	per cent
10/29/41	Dog B	22.6	22.1	-2.2
5/27/42	Dog I	18.5	17.6	-4.8
5/29/42	Dog J	13.7	14.4	+4.8
6/ 1/42	Dog G	20.6	20.4	-1.0
Mean.....				-0.8

a normal arterial saturation of 96 per cent, only three experiments indicated any appreciable change from this value; two experiments with sodium sulfadiazine resulted in reductions of 7.6 and 5.5 per cent while one experiment with sulfathiazole resulted in a reduction of 5.2 per cent.

DISCUSSION. Evidence that methemoglobin or sulfhemoglobin is produced in human subjects by administration of sulfanilamide in therapeutic doses is rather convincing in view of the results reported by Harris and Michel (5), Hartman, Perley and Barnett (6), Vigness and Spink (11), as well as Wendel, Wendel and Cox (13). However, the spectrophotometric work of Webb and Kniazuk (12) indicates that in rats receiving large doses of sulfanilamide a variety of blood effects resulted, some individual bloods containing methemoglobin

or sulfhemoglobin, some remaining normal and others containing an unidentified foreign pigment. Results obtained by administering sulfanilamide to dogs, as presented in this paper, are in general agreement with these workers in that considerable individual variation was also observed in dogs in regard to the production of these pigments. However, the dosage used by Webb and Kniazuk (2.0 grams/kilo-daily) was much greater than that used in the work reported here. No reports concerning the effect of sulfonamides, other than sulfanilamide, upon blood pigments have been available to the writer. In the dog with the dosage used (0.1 gram/kgm.) the other sulfonamides tested did not result in effects any different than those obtained with sulfanilamide.

Relatively little evidence except the work of King and Leslie (8) and Mull and Smith (10) is available concerning the effects of any sulfonamide, except sulfanilamide, upon the arterial oxygen saturation and capacity. With dogs there was considerable individual variation in response to a single therapeutic dose and this factor of individual response, as well as other experimental conditions, including particularly the dosage given, must all be considered in evaluating these apparently conflicting reports.

By employing a constant dosage and sampling the arterial blood at the peak concentration of the sulfonamide the maximum effect of single therapeutic doses upon the oxygen, and carbon dioxide capacity and arterial percentage saturation was measured. The oxygen capacity was used as an index of the blood's ability to transport oxygen since the formation of any pigment incapable of carrying oxygen would consequently reduce this value. As the results of the preceding tables indicate, there is some individual variation in regard to the effect upon oxygen capacity. There is no apparent correlation between blood level of the sulfonamide and its effect upon the oxygen, carbon dioxide capacity or percentage arterial saturation. Effects upon carbon dioxide capacity were also varied, due, to some extent, to use of both the sulfonamide as well as its sodium salt. Mean per cent changes in carbon dioxide capacity ranged from -5.6 volumes per cent for sodium sulfapyridine to $+4.8$ volumes per cent for sulfanilamide and sulfathiazole. These values may be considered as physiologically insignificant. Although Marshall, Cutting and Emerson (9), as previously mentioned, reported an acidosis in dogs resulting from sulfanilamide administration, Free, Davis and Myers (4) more recently find that therapeutic quantities of this compound do not cause marked changes in acid-base balance and believe the former workers used too large doses, causing marked respiratory and excretory dysfunction. The data reported in this paper are in agreement with those of the latter workers in that no marked effect upon the acid-base balance of the dog resulted when single therapeutic doses were administered.

The values of the arterial saturation obtained at peak levels of the blood sulfonamide concentration also failed to indicate any significant reduction resulting from the administration of these sulfonamides.

SUMMARY

Single therapeutic doses of the sulfonamide drugs (sulfanilamide, sodium sulfapyridine, sulfathiazole, sodium sulfadiazine, and sulfaguanidine) produced

small, but physiologically insignificant quantities of methemoglobin or sulf-hemoglobin in the blood of dogs.

Administration of single doses of these various sulfonamides resulted in a slight reduction in the blood oxygen capacity, although individual responses varied. The mean per cent reduction ranged from 4.1 per cent with sodium sulfadiazine to 9.5 per cent with sulfaguanidine.

The effect of these sulfonamides upon the carbon dioxide capacity (T_{40}) varied. Mean per cent changes in carbon dioxide capacity ranged from -5.6 volumes per cent for sodium sulfapyridine to a $+4.8$ volumes per cent for sulfanilamide and sulfathiazole.

The percentage arterial saturation was not significantly altered in fourteen experiments. In three experiments reductions of arterial saturation occurred, the maximum being 7.6 per cent.

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THE NERVOUS FACTOR IN SHOCK INDUCED BY MUSCLE TRAUMA IN NORMAL DOGS¹

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Traumatization of the muscle masses of the hind limbs has been used frequently as an experimental procedure for inducing shock in the dog. The extensive swelling of the traumatized legs indicates an appreciable local loss of fluid. In fact, Blalock and associates (1), Parsons and Phemister (2), and others, have presented good evidence that this local fluid loss is sufficient in itself to produce shock. However, other factors than simple loss of blood volume may also be contributing to the shock condition. O'Shaughnessy and Slome (3) showed that trauma applied to legs isolated from the body except for nerve connections produced fatal shock, a finding confirmed in essence by Lorber, Kabat and Welte (4). Also they observed that blocking the nerve pathways from the traumatized legs, as by spinal section or spinal anesthesia, delayed the onset of shock (3). A similar protective action of spinal anesthesia was noted by Meek (5) and by the writers (6) employing a different type of shock. Recently Freedman and Kabat (7) showed that when the local fluid loss following severe muscle trauma was curtailed by tight bandaging of the injured legs, shock still followed. The evidence would appear suggestive that nociceptive stimuli from the traumatized areas might be contributing significantly to the initiation of the shock condition. The problem is by no means settled, however, since several groups of investigators (8-10) have been unable to obtain evidence implicating the nervous system in shock following muscle trauma.

Several modified muscle trauma techniques have recently been advanced which, while still sufficient to cause fatal shock, do not necessitate breaking of bones and pulping of muscles. For instance, Kendrick, Essex and Helmholtz (11) and Best and Solandt (12) have used about 1500 rapid blows with a rubber mallet to the flexor muscles of each hind leg. The latter have concluded that the local fluid loss was not sufficient to account for the death of the animal. Neither, however, was there evidence that the shock produced was neurogenic in origin (13). Gregersen and co-workers (14)² have modified this technique even

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³ We are indebted to Dr. M. I. Gregersen for the opportunity of visiting his laboratory and witnessing an experiment. The blood pressure and blood concentration data presented in this paper for the control series of dogs are to be regarded as confirming as yet unpublished work first done in Doctor Gregersen's laboratory. We are concerned here primarily with the effect of nerve block upon shock.

further, so that a fewer number of blows, with a light mallet, delivered to all faces of the thigh muscles, produced fatal shock. This relatively mild muscle trauma procedure was adopted by the writers for a study of nervous influences upon the induction of shock.

METHODS. The traumatic procedure duplicated as nearly as possible that used by Best and Solandt (12) as modified by Gregersen (14). The animals were anesthetized with ether, the hair removed from the hind legs, and 400 to 800 blows, distributed over all faces of each thigh, delivered with a 200 gram rawhide mallet. The skin was bruised but not ruptured, and no bones were broken. Blood pressures were taken by frequent intra-arterial punctures, so that the number of blows could be governed by the level of blood pressure reached. The mean arterial pressure was reduced to 70 mm. Hg or lower before the trauma was stopped, for it has been our experience that when the post-trauma pressure was significantly above this level, fatal shock might not follow. Since in the animals subjected to spinal anesthesia prior to the trauma, the blood pressure was already reduced well below normal, the number of blows to be given could no longer be governed by the blood pressure level. An attempt was made to give these animals a greater degree of trauma than that required to produce fatal shock in the control animals. Likewise, in animals on which tourniquets had been placed prior to the trauma, the degree of trauma to be administered could be only estimated.

Following the trauma the animals were securely tied on their backs in a cradle, allowed to recover from the ether anesthesia, and maintained in this position until death occurred or for a total length of 7 hours. Hematocrit (capillary tube in air turbine), hemoglobin (Newcomer), and serum protein (falling drop) measurements were made at selected intervals.

I. Control series. In a series of 15 dogs subjected to leg trauma all but one developed fatal shock.⁴ The 14 (or 93 per cent) animals exhibiting shock died in 2 to 8 hours following completion of the trauma, the average interval being 4 hours. The procedure induced a decline from an average initial mean pressure of 116 mm. Hg to 64 mm. Hg. After completion of the leg pounding and upon recovery from anesthesia, the arterial pressure rose slightly to an average of 75 mm. Hg. This level was then maintained for several hours. A decline to 50 or 40 mm. Hg occurred some hours before death (fig. 1).

As shown by the representative protocols in table 1, hematocrit, hemoglobin and serum protein levels did not deviate significantly from the pre-trauma levels at any time during the experiment. These findings are in agreement with unpublished observations of Gregersen and co-workers (14).

II. Effect of position on the development of fatal shock. All animals in the control series were tied firmly on their backs throughout the experimental period—an admittedly most unnatural position for the dog. It seemed not only possible but probable, therefore, that such a position so long maintained might be actually conducive to the development of fatal shock. A second series of 12 dogs were

⁴ Recently the control series has been enlarged to a total of 37 dogs of which 33 or 89 per cent died in shock 4 to 9 hours after trauma.

traumatized in the usual manner, and then replaced in their cages immediately following trauma. Upon recovery from anesthesia, these animals sat or stood erect; some even walked about in their cages. Of this series of 12 dogs, 3 showed uneventful recoveries, the survival of 4 more was prolonged—even as much as 24 hours in one case, and the remaining 5 died in the usual four hour period. Those animals which recovered were allowed water after the eighth hour, which

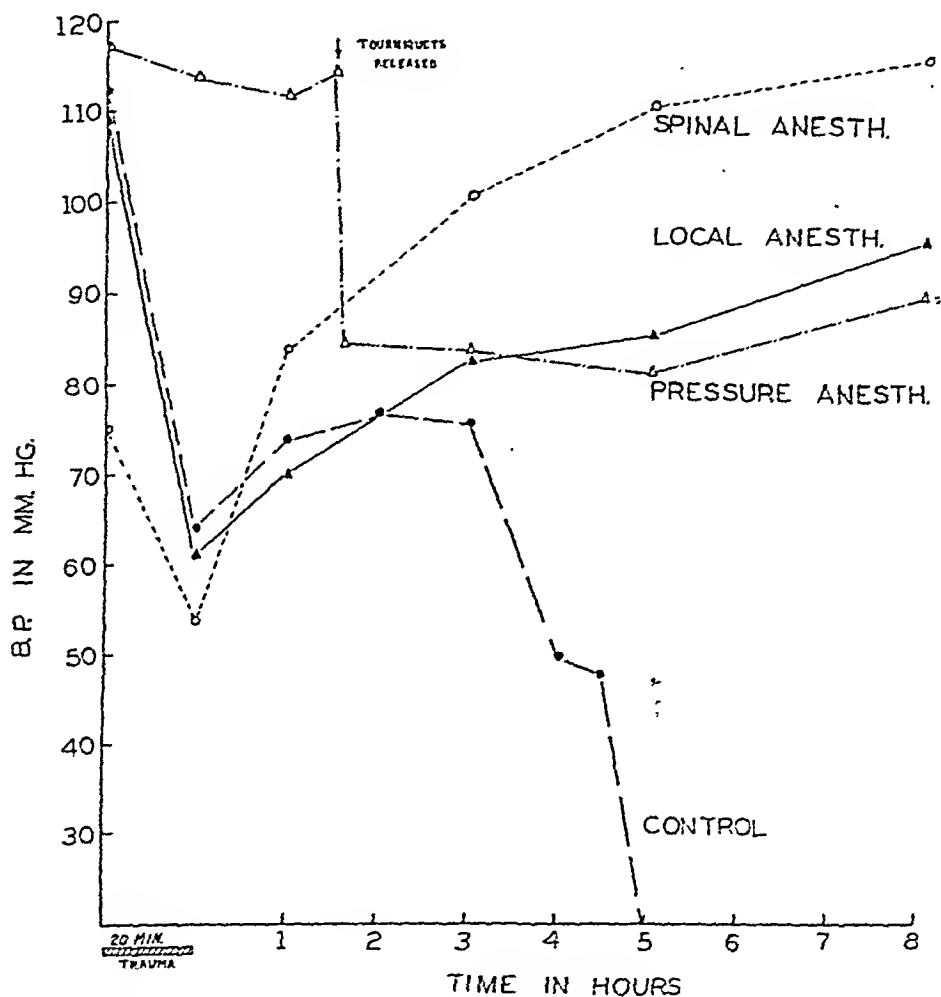


Fig. 1. Blood pressure changes following muscle trauma

they took avidly. There was a significant hemodilution by the twenty-fourth hour, which was to be expected considering the amount of water ingested.

The evidence is suggestive, therefore, that the severity of the shock was less in this series in which the animals were allowed to resume normal position after the trauma than in the previous series in which the animals were maintained on their backs.

III. *Effect of prolonged spinal anesthesia in preventing shock.* Evidence collected by several investigators suggests that muscle trauma to the legs may induce a flow of nociceptive stimuli from the injured area to the higher centers which contribute to induction of the shock state. An adequate level of spinal

anesthesia, especially if maintained over the critical hours following trauma, should block passage of nervous impulses and thereby prevent shock. To test this possibility a series of 12 dogs was placed under ether anesthesia, and just previous to leg traumatization, a dose of 1.8 to 3 cc. of a 2 per cent procaine solution, the exact amount being governed by the weight of the dog, was injected into the spinal canal usually at the level of the 3rd-4th lumbar vertebrae.

The ether anesthesia was discontinued following trauma, but additional procaine injections were made when a suggestion of leg or tail reflexes appeared. The spinal anesthesia was maintained for 3 to 4 hours, which usually required 2 to 3 additional injections. The dogs were kept on their backs, so that the mortality should have been of the order of 90 per cent or greater. Actually, 10 of the 12 animals showed none of the characteristic symptoms of shock and recovery was uneventful. Even the two animals which died were not clear cut failures, for both showed complications not associated with shock. The evidence was quite convincing that prolonged spinal anesthesia effectively prevents shock resulting from muscle trauma of the type described.

The arterial pressure was lowered from an average of 120 mm. Hg to 75 mm. Hg by the spinal anesthesia (table 1, fig. 1). The trauma caused an additional decline to an average of 54 mm. Hg. In the first hour after trauma, as with the control series, the pressure rose from this level to an average of 84 mm. Hg. Unlike the controls, however, the dogs given spinal anesthesia showed no secondary fall in blood pressure, and by the fifth hour the mean arterial pressure had attained normal levels (fig. 1). The dogs were removed from the cradle at the end of the seventh hour, placed in their cages and permitted to take water.

The dogs given spinal anesthesia showed significant increases in hematocrit, hemoglobin and serum protein levels. For example, the hematocrit level rose from an initial average value of 45.1 per cent to 47.8 per cent during the first hours following trauma, and to 49.7 per cent by the time the animals were replaced in their cages. After water was taken this concentration was rapidly lost. Serum protein concentration was not so marked but in the same direction. During the first two hours following the trauma, protein concentrations showed a rise from 6.51 to 6.73 grams per cent. There was little further change in the later hours of the experiment.

IV. *Effect of pressure anesthesia in preventing shock.* When the circulation in an extremity is completely occluded, as by a tight tourniquet, the limb is paralyzed both while the constriction is in place and for some time thereafter (15). The nerve block induced by such constriction, due in part to actual compression and in part to the effect of prolonged anoxia, we shall, for the sake of brevity, term "pressure anesthesia."

To test the effect of pressure anesthesia in preventing the fatal shock resulting from muscle trauma, heavy walled rubber tubing 12 mm. in diameter⁵ was tightly tied high around each hip. On the basis of a series of preliminary

⁵ It was erroneously stated (This Journal 138: 156, 1942) that the diameter of the tubing used as tourniquets was 120 mm. instead of 12 mm.

TABLE 1

*Prevention of shock following muscle trauma by means of prolonged spinal anesthesia, pressure anesthesia, and local procaine infiltration of the traumatized areas**

TIME	BLOOD PRES- SURE	PULSE	HEMATO- CRIT	HEMO- GLOBIN	SERUM PROTEIN	REMARKS
Dog. 1. 15.7 kgm. Control						
	mm. Hg	per minute	per cent	grams per cent	grams per cent	
10:00 a.m.	109	156	44.4	14.5	6.46	Under ether anesthesia
10:45 a.m.	72	120				Completed 500 blows to each leg
11:45 a.m.	75	130	43.1	14.5	6.63	
1:45 p.m.	49	196	44.1	14.6	6.83	Weak, extremities cold, died at 2:15 p.m.
Dog 2. 13.6 kgm. Spinal anesthesia						
9:45 a.m.	118	130	43.0	17.5	6.15	Under ether anesthesia. Given 2.5 cc. 2% procaine intraspinally
10:25 a.m.	55	132				Completed 500 blows to each leg
11:00 a.m.	101	144				Given 1 cc. procaine intraspinally
12:15 p.m.	123	148	54.1	22.3	7.35	Given 1.5 cc. procaine intraspinally
3:45 p.m.	120	184	52.7	25.2	7.35	Recovered from spinal anesthesia
9:30 a.m.	120	130	44.2	18.0	6.20	Took food, water, recovered
Dog 3. 11.8 kgm. Pressure anesthesia						
9:45 a.m.	134	216	45.1	14.5	5.99	Under ether anesthesia. Tourniquets placed on both hind legs
10:25 a.m.	142	180				Completed trauma of 550 blows to each hind leg
11:45 a.m.	80	144				Removed tourniquets
1:00 p.m.	90	180	48.2	15.7	7.24	
5:15 p.m.	85	148	55.5	18.1	7.17	Given water
9:45 a.m.	130	120	46.7	15.0	6.47	Appears normal, recovered
Dog 4. 9.1 kgm. Procaine infiltration of both hind legs						
10:00 a.m.	130	120	54.8	19.5	6.02	Injected 5 cc. 4% procaine into each leg
10:35 a.m.	70	97				Completed trauma of 500 blows to each leg
11:20 a.m.	76	100				Injected 2.5 cc. procaine into each leg
12:40 p.m.	83	140				Injected 2.5 cc. procaine into each leg
1:15 p.m.	98	170	55.6	19.9	6.19	Injected 2.5 cc. procaine into each leg
1:45 p.m.	103	176				Injected 2.5 cc. procaine into each leg
5:30 p.m.	108	164	56.0	20.0	6.46	Given water
9:30 a.m.	110	140	39.4	13.8	6.22	Appears normal, recovered

* Representative cases from each experimental group.

experiments, it was concluded that: *a*, the tourniquets must be applied some time before traumatization; *b*, that they would have to remain in place for 1 to 2 hours after the traumatization, if a nerve anesthesia sufficient to prevent the development of fatal shock was to be obtained. As a routine procedure, therefore, the tourniquets were applied 30 minutes before trauma, and maintained for a total period of 2 hours. Of 12 dogs so treated, 7 showed no symptoms of shock and recovered. Four more seemed well on the way to recovery when they were removed from the table after 7 hours, but showed sudden fatal collapse at about the 20th hour after the trauma. The survival period of these animals must be regarded as definitely prolonged. The single remaining dog died in shock 5 hours after trauma.

The blood pressure changes observed in animals subjected to pressure anesthesia differ quite distinctly from those of the control series. During the time the tourniquets were in place, when circulation through the traumatized legs was presumably completely occluded, there was no reduction from normal pressure levels. When, after $1\frac{1}{2}$ hours, the constrictions were removed, an abrupt pressure fall to about 85 mm. Hg followed within 2 to 3 minutes. It seems obvious that the initial pressure decline evoked by the leg trauma is directly related to the reduction in blood volume through sequestration of blood in the traumatized areas (fig. 1).

Once the pressure has fallen, upon release of constrictions, to 80-85 mm. Hg, there is little further change for a period of several hours, followed by a slow gradual rise to normal levels. Apparently the 2 hour interval during which the tourniquets were left in place was too brief. It must be remembered, however, that a constriction of both legs of dogs for 5 hours leads to an almost invariable fatal shock in itself (15). The tourniquet period could not be greatly increased, therefore, without actually contributing toward the severity of the shock.

The shock state following release of tourniquets is marked by an extreme rise in hematocrit, hemoglobin and serum protein levels, and a comparable fall in plasma volume (15). It was therefore only to be expected that the animals with tourniquets applied in conjunction with the muscle trauma should show blood concentration changes. For example, by the 5th hour after trauma, the hematocrit level had increased from an average initial value of 45.4 to 56.6 per cent, the hemoglobin from 15.4 to 18.6 grams per cent, and the serum protein from 6.2 to 7.1 grams per cent. There appeared to be no essential difference in the extent of these changes between the animals which died in shock and those which recovered (table 1).

V. *Effect of extensive procaine infiltration of the leg muscles in preventing shock.* An obvious and simple method for producing an anesthesia of the nerve elements in the traumatized legs would be a thorough infiltration with a local anesthetic of the area to be subjected to trauma. Procaine solutions of three strengths were used for this purpose, multiple injections into the thigh muscles, especially in the region of the major nerves, being made prior to the trauma and at 45 minute intervals. Local anesthesia was maintained for 3 to 4 hours, which required a total of 10 to 15 cc. of the procaine solution for each leg. In the first

3 animals, a 2 per cent procaine solution was used. Two of the three died in shock at the 6th and 11th hour after trauma, while the third showed an uneventful recovery. The strength of procaine solution was then increased to 3 per cent for 3 more dogs, two of which also died in shock, and the third recovered. For a series of 10 additional dogs, therefore, a 4 per cent procaine solution was employed. Seven of the 10 showed few if any signs of shock and recovered. At least two of the three animals which succumbed exhibited marked general systemic effects of a procaine overdosage.

Although the rise in blood pressure from the level reached after trauma was relatively slow (fig. 1), it was, in those animals which recovered, uninterrupted until normal pressures had been reached. There was a small increase in hematocrit (from 52.2 to 55.2 per cent), in hemoglobin (17.8 to 19.0 grams per cent) and in serum protein (6.5 to 7.0 grams per cent) in the first hours following trauma. These changes were in the same direction as those observed in the dogs given spinal anesthesia (table 1).

DISCUSSION. The muscle trauma procedure employed produced fatal shock in 93 per cent of the control animals.⁴ The survival period was never longer than 9 hours, and was usually about 4 hours. The muscle bruising leads to a pooling of blood in the injured areas, despite the absence of hematocrit change. The evidence appears suggestive that the precipitous decline in blood pressure during the actual traumatization is a consequence of this local fluid loss. At least, there is no pressure reduction so long as the circulation to the bruised legs is occluded by means of tight tourniquets, and an abrupt pressure fall does follow release of those constrictions.

Another additional factor would be that suggested by several workers, viz., that injury to nervous structures in the traumatized areas may be sufficient to induce a general circulatory failure. The data presented here afford good evidence for the reality of the nervous factor in shock which follows the type of trauma used. Prolonged spinal anesthesia, which should prevent nociceptive stimuli from the injured legs from reaching the higher centers, not only prevented all symptoms of shock but allowed indefinite survival in 83 per cent of the cases.

The obvious criticism that because the spinal anesthesia itself lowered the blood pressure, the local fluid loss resulting from the given amount of trauma would be less extensive than in the control animal with normal pressure level, seems met by the experiments where local blocking of the nerves was obtained either by means of pressure anesthesia attendant upon the application of tight tourniquets, or through infiltration of the area to be injured with a strong procaine solution. In both, the blood pressure was at normal levels prior to trauma. It is true that neither of these procedures could give a protection against shock as complete as that afforded by spinal anesthesia. For example, a two hour period of constriction was sufficient to allow successful recovery in 58 per cent of the cases, and definitely prolonged the survival period of 33 per cent more. Prolonged anesthetization of the limb by frequent injections of procaine prevented shock in 70 per cent of the cases.

The rapid local fluid loss which follows the release of tourniquets after a longer

period of time, and the general systemic effects of procaine overdosage which followed the use of relatively large doses of procaine, served as critical limiting factors for the two procedures. It is quite possible, therefore, that the lower protection of the two measures against shock, as compared with that furnished by spinal anesthesia, was merely a reflection of an inadequate anesthesia level. However, the fact that either pressure or local anesthesia can prevent circulatory failure in a good proportion of cases, and that spinal anesthesia affords almost complete protection against shock, seems good evidence for the existence of a nervous factor in this type of shock.

Probably neither nociceptive nervous stimuli nor local blood loss, considered separately, are sufficiently damaging to induce the severe shock characteristic of the type of muscle trauma employed in these experiments. However, when the effects of the nervous factor are superimposed upon the local fluid loss and resulting blood volume decline, fatal shock almost invariably results.

SUMMARY

1. Traumatization of the muscles of both hind legs by 800 to 1600 blows with a light rawhide mallet, in which the skin was not ruptured nor bones fractured, produced fatal shock in 14 of 15 control dogs. The survival periods ranged from 2 to 8 hours, with an average of 4 hours after the completion of the trauma.

2. When the animals were replaced in their cages to resume a normal posture after the trauma, instead of remaining tied on their backs in a cradle, the mortality of a series of 12 dogs was reduced to 75 per cent, and 4 of this series showed a definite prolongation of the survival period.

3. Spinal anesthesia, maintained for 3 to 4 hours, prevented all symptoms of shock and allowed uneventful recoveries in 10 of 12 animals.

4. A local anesthesia of the legs by means of pressure (tight tourniquets) maintained for a 2 hour period, protected 7 of 12 dogs against shock, and markedly prolonged the survival of 4 more.

5. Thorough infiltration of the areas to be traumatized with a 4 per cent procaine solution, repeated frequently over a 3 to 4 hour period, prevented fatal shock in 7 of 10 dogs.

6. The evidence indicates that a flow of nociceptive stimuli from the traumatized regions, unless prevented by spinal anesthesia or a local block, is an important contributing factor in the initiation of the shock state which follows the type of muscle trauma employed.

7. The nervous factor taken in conjunction with the loss of blood into the injured area almost invariably produces shock, but it is improbable that either factor considered apart from the other, is sufficient to induce a fatal outcome in the type of trauma employed.

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CHANGES IN THE RENIN-ANGIOTONIN SYSTEM IN HEMORRHAGIC SHOCK^{1, 2}

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The development of tachyphylaxis to renin following prolonged, severe hemorrhagic hypotension was observed in a previous study (1). We suggested that the impaired responsiveness to injected renin was due to exhaustion of renin-substrate (hypertensinogen, renin-activator) resulting from the liberation of relatively large amounts of renin by the animal's own kidneys. It was known that in hemorrhage the kidney is responsible for the appearance of a renin-like pressor substance in the blood, which acts as a compensatory mechanism to sustain blood pressure (1, 2, 3, 4). The subsequent development of tachyphylaxis to renin was, therefore, considered as a possible contributory factor in the development of shock. The present investigation confirms our previous suggestion.

METHODS. *Preparation of samples.* Samples from the cannulated femoral artery were immediately centrifuged for a standard time. Care was taken to avoid even slight coagulation. The importance of such care in the perfused rabbit's ear has been emphasized by Landis, Wood and Guerrant (5). Furthermore, defibrinated blood or serum contracts isolated intestine (6, 7). The sample was withdrawn directly into a dry syringe containing liquid Connaught heparin (20 to 40 units per cc. of blood). It may have been unnecessary to exclude coagulation in our samples, which were heated, since Greeley (6) states that boiling destroys the power of defibrinated blood to contract intestine. Precautions were taken against hemolysis. In order to minimize changes occurring *in vitro* in the renin-angiotonin system, the samples were prepared at once. Plasma for the assay of endogenous angiotonin (hypertensin) was heated for 6 minutes in a water bath at 90°C. to destroy all known components of the renin-angiotonin system except the thermostable angiotonin. Acetic acid to make a final concentration of 0.12 per cent was added. After mixing, the fluid portion was separated by centrifugation. In the determination of renin-substrate (hypertensinogen) a standard quantity of renin was added to the plasma, and the mixture was incubated for 6 minutes at 38°C. in a thermostatically controlled water bath. The subsequent procedures were the same as described for angiotonin, and renin-substrate was estimated by the amount of angiotonin formed. A short period of incubation was selected in order to minimize destruction of hypertensin (angiotonin) by hypertensinase (8). Except in early experiments renin available from the S. M. A. Corporation (hog renin extract no. 1000 containing 10 pressor units per cc.) was employed in amounts of 0.04 cc. per cubic

¹ This investigation was aided by a grant from the John and Mary R. Markle Foundation.

² A preliminary report has been presented in *Federation Proceedings* 2: 7, 1943.

centimeter of plasma. This concentration of renin was an excess, for doubling the amount did not increase the yield of angiotonin from normal dog's plasma. Control tests showed that the renin did not contain sufficient hypertensinase to influence the assays.

In a few experiments in the determination of hypertensinogen (renin-substrate), preliminary destruction of hypertensinase was accomplished by the method of Leloir, Muñoz, Braun-Menendez and Fasciolo (9). Immediately after adding renin, the pH of the plasma was adjusted to 4.2 using brom phenol blue. After 20 minutes at 25°C. and subsequent return of the pH to about 7.5, the plasma was incubated for 15 minutes at 38°C., and then heated at 90°C. for 6 minutes.

All samples were refrigerated until time for bio-assay, which was usually performed on the following day. The pH was adjusted by means of phenol red to approximately 7.5 immediately preceding assay. This completely prepared material will be referred to subsequently as *prepared sample*.

Inactivation of adrenalin. Adrenalin affects all the test objects used in this study, namely, the perfused rabbit's ear, the intact animal, and the ileum of the guinea pig. Adrenalin when added to angiotonin reduced the response from the ileum. Also adrenalin in plasma was resistant to a temperature of 90°C. for 6 minutes.

An attempt therefore was made to inactivate adrenalin in the prepared samples. Hydrogen peroxide was unsuitable, for it (or possibly the contained preservative) affected the ileum adversely. Formaldehyde is known to inactivate adrenalin (10). Adrenalin added to fluid obtained from normal heated plasma was inactivated in less than one hour by formaldehyde in a concentration of 40 γ per cc. (experiments on the intact animal and on the perfused ear). Furthermore, the reduction by adrenalin of the response of the ileum to angiotonin (in the prepared sample for the assay of renin-substrate) was abolished by formaldehyde. None of the 3 test objects was affected adversely, nor did they reveal any inactivation of angiotonin even after its exposure to formaldehyde for about 5 hours. Potential endogenous adrenalin, therefore, was inactivated in the prepared samples by the addition of formaldehyde (40 γ per cc.) an hour or more before assay.

Methods of bio-assay. In tests on the ileum of the guinea pig many of the recommendations made by Code (11) for the assay of histamine were followed. The intestinal segment was immersed in a volume of approximately 15 cc. of Tyrode's solution (containing 0.2 per cent dextrose). The solution was aerated with oxygen, and was kept at 38°C. by a thermostatically controlled, stirred water bath. Since atropine sulphate was employed (1.0 γ per cc. of Tyrode's solution), there was little or no rhythmic activity, and comparison of responses was greatly facilitated. One cubic centimeter of the prepared sample was adequate for a single test of endogenous angiotonin, and 0.1 to 0.2 cc. for the assay of renin-substrate. The latter samples were diluted 1:5 or 1:10 with Tyrode's solution. All material was warmed to 38°C. before testing. Multiple tests were made of each sample, and usually one sample was chosen as a standard for comparison with others of the same type. The loading of the intestine was sometimes

changed between assays of different types of samples. While this method required a rigidly controlled technique, it yielded reproducible results, and was more sensitive and dependable than the other types of bio-assay.

The results are expressed in the tables as the height of the excursion of the lever on the drum, and are in most instances averages of several responses. Although the sensitivity of the muscle varied, the results expressed in this manner closely approximated the finer comparisons always made between crucial samples. It must be emphasized that the data should be considered only for directional and rough quantitative changes.

In some experiments the intact animal was used for assay. Details will be given later.

Assays also were made on the isolated rabbit's ear perfused by the technique of Page (12) with the Ringer-Locke's solution (containing calcium) recommended by him. The amount of prepared sample injected was 0.2 cc. Several ears were employed for each experiment.

Procedures in dogs. Unless otherwise noted the dogs were anesthetized with morphine and sodium barbital as advised by Wiggers (13). Mean arterial blood pressure was recorded from the carotid artery with a mercury manometer. Although variations were introduced in many experiments, the procedure outlined by Wiggers and his associates (14, 15, 16) for the production of hemorrhagic shock was generally followed. The animals usually were subjected to a period of moderate hypotension (ca. 50 mm. Hg) and to a subsequent period of severe hypotension (ca. 30 mm. Hg). When necessary, the animal's own blood containing 5 units of Connaught heparin per cubic centimeter was reinfused at body temperature. Volumes of blood will be expressed as cubic centimeters per kilogram of body weight. Since large amounts of blood were required for samples, demonstration of irreversibility by reinfusion of all withdrawn blood was not possible.

Bilateral nephrectomy and bilateral adrenalectomy were performed through the retroperitoneal approach. Adrenalectomy when purposely accompanied by light anesthesia and by excessive stimulation of surrounding nervous tissue will be called *traumatic adrenalectomy*. In other cases deeper anesthesia was used, nervous stimulation was minimized, and electro-coagulation was employed.

RESULTS AND DISCUSSION. I. *Assays on Ileum.* A. *Experiments on nephrectomized dogs.* In 5 of the 7 control experiments on bilaterally nephrectomized dogs, possible complications in the assay due to adrenalin were avoided either by treatment of the samples with formaldehyde or by bilateral adrenalectomy. Essentially the same results were obtained regardless of control of adrenalin. In spite of severe prolonged hypotension, there was a progressive and marked increase in renin-substrate in all except one of the animals (table 1). In dogs without hemorrhage an increase of hypertensinogen (renin-substrate) has been observed 48 hours following nephrectomy (8). It is of interest that we observed definite increases in these bled dogs at earlier times (4 hrs. in one case).

In experiment 26 there was an early and progressive decrease in renin-substrate. The anesthesia used in this experiment was morphine and chloralose. Apparently under certain conditions renin-substrate may diminish in the absence of the

TABLE 1

Assays of plasma from bled nephrectomized dogs

Periods of blood pressure are placed in consecutive order. The greatest total loss of blood existing at any one time in a period and the blood returned in that interval are given. The response of the ileum obtained in the assay of renin-substrate (RS) or angiotonin(A) refers to a sample taken at the end of the corresponding period.

EXP. NO.	BLOOD P	TIME	MAX. BLOOD LOSS	BLOOD RE-TURNED	ILEUM		EXP. NO.	BLOOD P	TIME	MAX. BLOOD LOSS	BLOOD RE-TURNED	ILEUM		
					RS	A						RS	A	
Nephrectomized dogs														
21	mm. Hg	min.	cc. per kgm	cc. per kgm.	mm.	mm.	26	mm. Hg	min.	cc. per kgm.	cc. per kgm.	mm.	mm.	
	Nephrectomy							Nephrectomy						
	120		0	0	25	4		131		0	0	30	2	
	83-120	70	25	0				90-131	43	20	0			
	61- 62	56	30	0	30	2		46- 68	31	30	0	20		
	38- 52	34	35	0	31	6		30- 50	207	43.2	15	13		
	16- 20	10	39	0	41	4		12- 32	40	32.3	13.5	10	1	
12- 24	40	43	0	51	3	Morphine-chloralose; marked intestinal lesions								
Nephrectomized dogs (adrenalin inactivated with formaldehyde)														
78	100		0	0	31	15	81	116		0	0	14	27	
	Nephrectomy							Nephrectomy						
	99		5.3	0	37	15		98		5.6	0	22	20	
	60- 96	41	30	0				86-102	67	30	0			
	40- 60	134	33	4	27	25		24- 56	93	35	8	60	31	
	14- 36	35	34.3	9.3	59			22- 38	98	33.4	8.4	78	20	
	Last sample taken at death; mild intestinal lesions							Marked lesions						
Nephrectomized adrenalectomized dogs														
52	123		0	0	34	0	50	103		0	0	37	22	
	Nephrectomy and adrenalectomy							Nephrectomy and adrenalectomy						
	72-122	26	25	0				81- 92	63	4.4	0		24	
	34- 47	63	30	9		1		30- 66	94	10	0			
	44- 48	41	23	4.5		0		25- 30	31	10	0	49	22	
	40- 43	44	20.5	0		1		16- 22	17	16.6	4.2			
	26- 34	63	22.5	7	64	1		16- 25	5	14.6	0	63		
Marked lesions						Marked lesions								
27	Nephrectomy and adrenalectomy													
	130		0	0	21	2								
	89- 91	50	10	0	26	2								
	79- 81	12	13	0										
	50- 68	45	23	0	23	2								
	23- 40	18	30	0	51	1								
	Morphine-chloralose; no lesions													

kidneys. It is possible that severe hypotension may disturb the production or mobilization of this constituent.

The usual increase in renin-substrate probably explains the average potentiated response to injected renin obtained in nephrectomized dogs following hemorrhagic hypotension in our earlier study of tachyphylaxis (1). Experiment 26 is of interest because in this previous study some animals failed to show potentiation.

Assays of endogenous angiotonin revealed no significant change (table 1). Relatively constant nonspecific responses were observed. These were large in 3 experiments due to unusually light loading of the ileum. A small increase of 10 mm. occurred in one instance, but an isolated change of this magnitude is of doubtful significance. One sample, taken at death after the administration of adrenalin and artificial respiration, gave a markedly increased response. Assays of angiotonin from such samples are questionable, and have been routinely discarded. It should be noted that none of the samples was obtained more than 7 hours following nephrectomy. These findings present a striking contrast to those obtained from intact animals, which with but few exceptions showed marked initial increases (tables 2-5).

B. *Experiments on dogs retaining their kidneys.* Ten of the 15 intact dogs and 3 of 10 adrenalectomized dogs showed significant reductions of renin-substrate (hypertensinogen) below the initial level (tables 2-5, fig. 1). The preliminary increase, which often occurred following hemorrhage, will be discussed later. Our data do not permit an accurate evaluation of the minimum effective intensity and duration of hypotension necessary to reduce renin-substrate invariably. There was a wide variation among dogs. In experiment 73 (table 3) renin-substrate was exhausted after a hypotension at 30 to 58 mm. Hg lasting 106 minutes. In experiment 77 (table 3) renin-substrate was not decreased despite a moderate hypotension (45-56 mm. Hg) lasting 98 minutes and a subsequent drastic hypotension (25-33 mm. Hg), 157 minutes in duration. In experiment 30 (table 2) mild hypotension for about 10 hours did not deplete renin-substrate. An initial period of moderate hypotension (ca. 50 mm. Hg) lasting about 90 minutes was not generally adequate. A further period of more intense hypotension was required. Reduction in renin-substrate was not related to the amount of blood lost (in cubic centimeters per kilogram of body weight). The above findings rather closely parallel those obtained by Wiggers and his collaborators with regard to the development of irreversible hemorrhagic shock (14, 15, 16). At autopsy, most of the animals showed the characteristic lesions of the mucosa of the upper intestines, emphasized recently by Wiggers.

Four dogs were subjected to a preliminary single bleeding of 20 to 25 cc. per kgm. After an interval of $15\frac{1}{2}$ to $20\frac{1}{2}$ hours angiotonin was not demonstrable, while renin-substrate, in the 2 cases where measurements were made, was markedly increased. The results following subsequent bleeding and hypotension were most interesting. Three of the animals showed unusually early reductions in renin-substrate or endogenous angiotonin (table 5). Reduction in angiotonin, as will be pointed out later, follows depletion of renin-substrate. For example, in experiment 34 serious depletion of renin-substrate occurred after 58 minutes of hypotension at 38-56 mm. Hg.

It did not seem probable that endogenous adrenalin was likely to interfere in

assays of renin-substrate. Only 0.1 to 0.2 cc. of the prepared sample was used for a test, and the amount of angiotonin relative to adrenalin should have been enormously increased by the incubation with renin (assuming the presence of

TABLE 2

Assays of plasma from bled intact dogs (no inactivation of adrenalin)

The arrangement and symbols are like those of table 1. Determinations of renin-substrate involving destruction of hypertensinase are listed under RSh. Experiments in which a balloon was introduced into the incised small intestine either by opening the abdomen (*balloon*) or by incising a Biebl loop (*Biebl*) are indicated.

EXP. NO.	BLOOD P	TIME	MAX. BLOOD LOSS	BLOOD RE-TURNED	ILEUM			EXP. NO.	BLOOD P	TIME	MAX. BLOOD LOSS	BLOOD RE-TURNED	ILEUM	
					RS	RSh	A						RS	A
	mm. Hg	min.	cc. per kgm.	cc. per kgm.	mm.	mm.	mm.		mm. Hg	min.	cc. per kgm.	cc. per kgm.	mm.	mm.
23	125		0	0	23		6	22	112		0	0	33	3
	34-60	97	30	3	22		31		80-98	192	40	0	47	42
	22-38	83	31	13	2		15		62-86	33	45	0	56	
	Morphine-chloralose; pregnant; marked intestinal lesions								60-90	75	50	0		35
									45-46	45	55	0	63	40
24	118		0	0	46		1	30	124		0	0	71	7
	63-118	60	34	0	58		19		100-134	175	40	0		45
	40-88	78	47	0	54		27		64-116	457	43	0	77	43
	18-60	122	52	29	2		13		38-72	148	45	0	74	45
	Morphine-chloralose; marked lesions								Mild intestinal lesions					
43	129		0	0	98	78	7	36	99		0	0		17
	96-118	25	15	0					78-93	23	15	0		
	38-68	117	34	0			13		38-53	57	20	0		65
	32-54	55	39	2			53		37-59	68	26	4.6		
	32-38	25	43	0					20-35	54	29	9.6		
	76-90	175	43	30.5					40-82	88	21.4	9.8		49
	24-48	42	29.5	4					Balloon; marked lesions					
	68-76	113	25.5	13	7	14	3							
	Pentothal-barbital; balloon; mild lesions													
41	130		0	0		64		49	109		0	0	44	0
	44-72	40	20	0					79-108	38	35	0		
	38-55	94	31	0					35-67	25	43	0		
	31-42	26	34	3					37-58	103	54	0		23
	31-120	150	31	24		90			33-35	40	56	0		
	Pentothal-barbital; balloon; no lesions								18-28	42	58	0	20	36
									Biebl; moderate lesions					

renin-substrate). However 18 experiments were performed in which adrenalin was either inactivated by formaldehyde or eliminated by bilateral adrenalectomy (tables 3 to 5). Significant reductions in renin-substrate were observed in 5 intact and in 3 adrenalectomized dogs. Furthermore prepared samples treated

TABLE 3

Assays of plasma from bled intact dogs (adrenalin inactivated by formaldehyde)

The abbreviations of the preceding tables are used. Assays made on the intact cat are included

EXP. NO.	BLOOD P	TIME	MAX. BLOOD LOSS	BLOOD RE-TURNED	ILEUM		CAT		EXP. NO.	BLOOD P	TIME	MAX. BLOOD LOSS	BLOOD RE-TURNED	ILEUM	
					RS	A	RS	A						RS	A
	mm. Hg	min.	cc. per kgm.	cc. per kgm.	mm.	mm.	mm. Hg	mm. Hg		mm. Hg	min.	cc. per kgm.	cc. per kgm.	mm.	mm.
73	134		0	0	38	0			70	123		0	0	57	1
	119-132	25	25	0						58-138	61	40	0		
	68- 94	28	40	0						28- 60	99	45	2		10
	30- 58	106	45	12	0	31				25- 40	51	46	2	92	13
	Moderate intestinal lesions						Moderate intestinal lesions								
75	133		0	0			35	0	77	134		0	0	25	4
	110-134	29	30	0						86-123	215	50	0		
	24- 88	164	40	20			33	19		45- 56	98	55	0	37	16
	18- 42	34	41.7	8.7			30	18		26- 33	67	58.3	0	24	31
	Mild lesions						Mild lesions								
76	134		0	0	51	3	43	5	72	110		0	0	44	7
	104-131	37	40	0						62-108	80	35	0		
	34- 70	103	50	2	25	17	30	43		30- 62	30	35	0	50	20
	22- 38	37	57.1	7.1	12	13	12			17- 61	197	37.5	13.5	15	42
	Minimal lesions						Marked lesions								

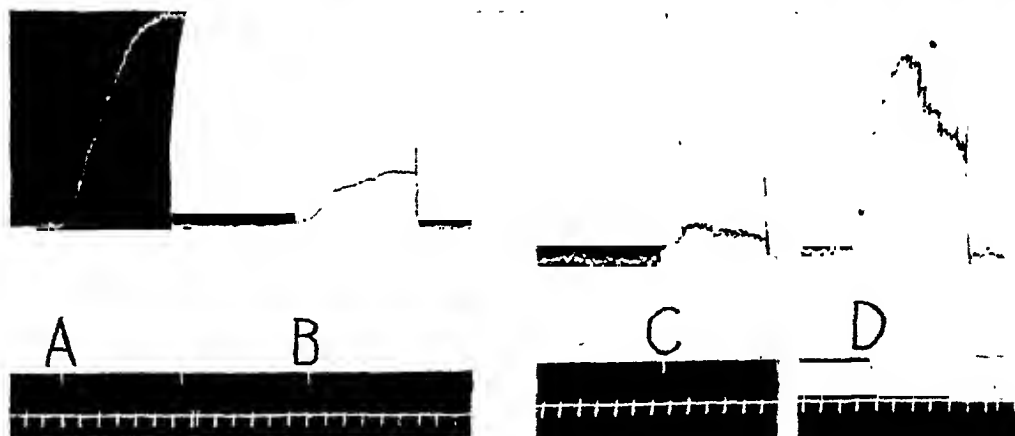


Fig. 1. Examples of responses of the ileum to prepared samples from experiment 72 (table 3). Adrenalin inactivated with formaldehyde.

Renin-substrate assay: A = initial sample; B = final sample; dose—0.14 cc. diluted to 0.7 cc.

Angiotonin assay: C = initial sample; D = final sample; dose—1.0 cc. Time interval—30 seconds.

with formaldehyde and showing reductions in renin-substrate elicited the same responses from the ileum as the corresponding untreated samples. It is concluded that adrenalin did not interfere significantly in the assay of renin-substrate.

TABLE 4

Assays of plasma from bled adrenalectomized dogs

The arrangement of the preceding tables is followed. The determinations of renin-substrate involving destruction of hypertensinase and assays on the intact dog are included.

[illegible]

In many of the experiments it was necessary to return blood. It might be supposed that the observed decreases in renin-substrate were due to changes which had occurred in this blood. However in some experiments diminutions occurred without the return of any blood; in others no diminution occurred despite the return of blood. Moreover, assays of blood returned showed that

TABLE 5

Assays of plasma from bled intact dogs subjected to a preliminary hemorrhage

The symbols of the preceding tables are employed. RSpH indicates determinations of renin-substrate in which the pH was adjusted with the glass electrode. The dogs were narcotized with morphine for the preliminary bleeding.

EXP. NO.	BLOOD P	TIME	MAX. BLOOD LOSS	BLOOD RE-TURNED	ILEUM			EXP. NO.	BLOOD P	TIME	MAX. BLOOD LOSS	BLOOD RE-TURNED	ILEUM		
					RS	RS _{pH}	A						RS	A	
No inactivation of adrenalin															
34	mm. Hg	min.	cc. per kgm.	cc. per kgm.	mm.	mm.	mm.	33	mm. Hg	min.	cc. per kgm.	cc. per kgm.	mm.	mm.	
			0	0			0					0	0		4
			20	0								20	0		
	Interval of 20½ hours								Interval of 20½ hours						
	120		20	0	103		0		126		20	0		7	
	92-122	60	45	0					68-126	102	40	0		22	
	38- 56	58	50	0	18		95		29- 51	49	41.1	0		33	
	56- 75	15	53.1	8.1			74		Adrenalin (0.2 cc. 1:10,000)						
34- 55	17	46.6					106-143	37	42.7	18.1		16			
20- 37	150	46.6	14	0		0	42- 82	46	26.2	0					
							24- 42	76	26.2	0		17			
Marked intestinal lesions															

Adrenalin inactivated by formaldehyde

80			0	0	39		13	74			0	0	69	3
			20	0							25	0		
	Interval of 15½ hours								Interval of 19 hours					
	112		20	0	54	54	14		112		25	0	112	2
	78-118	95	45	0					112-113	18	35	0		
	58-78	13	45	0					34-58	106	52	0	41	21
	35-60	90	58	0	43	48			17-30	41	55.3	0	11	24
	20-31	70	63	2	46	41	32		22-32	96	58.6	23.6	2	5
	23-37	112	65.8	20.8	12	13	46		Moderate lesions					
	Moderate intestinal lesions													

its content of renin-substrate was not diminished. Instead it was increased probably due to the fact that part of the blood had been collected during the early period when renin-substrate was increased. With few exceptions samples were not collected less than 20 minutes following the return of blood.

It seemed possible that increased amounts of hypertensinase, by causing excessive destruction of hypertensin (angiotonin) during the incubation with renin,

might have been responsible for the reductions observed in the assay of hypertensinogen (renin-substrate). Although plasma contains only a minimal amount of hypertensinase (17, 18), hemolyzed erythrocytes contain about 100 units per gram (17). Progressive hemolysis was observed frequently in our dogs, particularly following the return of blood. Accordingly measurements of hypertensinogen involving preliminary destruction of hypertensinase were made. The potency of these samples was greater, and smaller dosage or heavier loading of the ileum was employed. The results in general paralleled those obtained from samples prepared in the usual manner (tables 2 and 4).

Since the optimum pH for the activity of renin lies between 7.5 and 8.5 (8), changes in the blood toward the acid side, known to occur in hemorrhage (19), might have diminished the yield of angiotonin during the incubation with renin, and hence have given an apparent decrease in renin-substrate. The experiments just cited, in which hypertensinase was destroyed, discount this possibility since the pH was adjusted to about 7.5 prior to incubation with renin at 38°C. Other experiments in which the pH of the plasma was adjusted by means of indicators also suggested that this factor was of no significance. One experiment was performed using the glass electrode at 28°C. Portions of the samples of plasma were adjusted to a pH within 0.02 of 7.6. Assays made with adjusted and unadjusted samples gave similar results (table 5, expt. 80).

Thus the diminutions observed in renin-substrate are not artifacts. The observed depletions correlate well with previous observations on the development of tachyphylaxis following hemorrhage. In this previous study (1) test doses of renin were given prior to hemorrhage, and morphine and chloralose were used as an anesthetic. These facts may explain the development of tachyphylaxis following hypotension less severe or less prolonged than usually was required to reduce renin-substrate in the present experiments.

Why does a reduction in renin-substrate occur? Exhaustion by endogenous renin appears to be the most obvious and important factor. Severe hypotension may interfere with the production or mobilization of renin-substrate. Shifts of fluid or protein between plasma and interstitial fluid are likely to be involved. Finally there remains the possibility that changes in hypertensinase or in other less well-known factors operating *in vivo* may be concerned. Diminution of renin-substrate may have significance in relation to the treatment of shock.

In almost half of the animals an early rise in the concentration of renin-substrate was observed, and in the remainder this change may have been missed by failure to take samples at the proper time. The reason for this interesting phenomenon is obscure. It might be ascribed to increased endogenous angiotonin in the samples. This explanation does not seem entirely adequate in view of the respective amounts needed for the assay of endogenous angiotonin and renin-substrate (1.0 cc. as against 0.1–0.2 cc.). Moreover elevations of renin-substrate occurred before significant increases in angiotonin were demonstrable (tables 4 and 5). Increase in renin-substrate possibly may serve as a compensatory mechanism heightening the effectiveness of renin.

Changes observed in endogenous angiotonin may be seen in tables 2–5 and

are illustrated in figure 1. With but few exceptions, angiotonin was demonstrable after the period of moderate hypotension (ca. 50 mm. Hg). Increases also were observed following relatively mild or short hypotension. For example, in experiment 30 (table 2) a marked increase was apparent although the blood pressure had not been below 100 mm. Hg (the blood loss was 40 cc. per kgm. of body weight); in experiment 24 (table 2) angiotonin was demonstrated after a period of 60 minutes during which the blood pressure had been reduced from 118 to 63 mm. Hg. We have made no attempt to determine threshold values.

The effect of adrenalectomy upon the behavior of the renin-angiotonin system is an interesting problem. It is possible that the secretion of adrenalin in hemorrhage or in other conditions enhances the output of renin by altering renal circulation. In half of the adrenalectomized animals, the rise in angiotonin was small or absent (table 4). In the remainder, however, apparently normal changes occurred. The data also suggest that depletion of renin-substrate occurs less readily than in normal dogs. A final decision of this interesting question must await further investigation. Little hemorrhage was required to produce the hypotension in dogs subjected to traumatic adrenalectomy, and yet diminution in renin-substrate and increase in angiotonin could occur.

In some cases after the initial increase, there was a secondary decrease in endogenous angiotonin. The data indicate that this fall follows depletion of renin-substrate. The possibility for interference by endogenous adrenalin appears greater in assays of angiotonin than in those of renin-substrate. However, in experiment 74 (table 5), in which adrenalin was inactivated by formaldehyde, a marked secondary decrease occurred. In the same experiment assays of the last 2 samples made with and without formaldehyde yielded identical results. That a decrease in angiotonin (hypertensin) should result from exhaustion of renin-substrate seems obvious. Other factors, such as increase of hypertensinase, also may be concerned.

II. *Other Methods of Assay.* Assays were made on intact animals by determination of the change in blood pressure resulting from the injection of the undiluted prepared sample into the femoral vein. The responses obtained were angiotonin-like in character. Possible complication from adrenalin was avoided by adrenalectomy or by formaldehyde. In assay on the cat atropine sulphate was employed ($\frac{1}{2}$ or 1 mgm. per kgm. of body weight subcutaneously), and volumes of 1 cc. and 10-15 cc. respectively were used in the assay of renin-substrate and angiotonin. Determinations of renin-substrate were done at least in duplicate. Assays of angiotonin also were made on the intact dog using a volume of 15 to 20 cc. for injection and in this case simultaneously withdrawing the same volume of blood from the femoral artery. Difficulty was experienced in obtaining significant responses in the assay of angiotonin particularly in the dog. Increase in angiotonin and depletion of renin-substrate were demonstrated (tables 3 and 4). The results confirmed those obtained on the ileum with the same material.

Corroborative evidence was obtained with the perfused rabbit's ear, although in our hands this method proved less reliable and less sensitive than the ileum.

Frequently changes demonstrable by the latter were not detected by the ear. Otherwise, parallel results were obtained by the two methods. In the case of the ear, determinations of renin-substrate were more satisfactory than those of angiotonin.

Assays were made on the ear in the 3 experiments in which both bilateral adrenalectomy and bilateral nephrectomy had been performed. No increase in vasoconstrictor activity was observed in samples prepared for the assay of angiotonin. In all 3 experiments increases of renin-substrate, paralleling those shown by the ileum, were obtained. In experiment 26, performed on a nephrectomized animal retaining its adrenals, a decrease of renin-substrate was indicated by both methods.

The ear was used in 3 of the experiments performed on intact dogs and involving the use of formaldehyde. In 2 instances increases of angiotonin were observed. In the third experiment no increase was detected although it was demonstrated by the ileum. Decreases in renin-substrate were observed in all 3 experiments, and confirmed assays on the ileum.

The ear also was employed in the experiments on adrenalectomized animals. Increases of angiotonin, confirming those demonstrated by the ileum, were obtained in 2 of the 6 experiments in which observations were made. In 4 instances no increase was demonstrable, but the increases in the responses of the ileum were either small (14 mm. or less) or absent. Assays of renin-substrate were made on the ear in 6 of the experiments. In 2 instances increases in renin-substrate were observed, and confirmed assays on the ileum. In 3 experiments no change could be detected, but either no alteration or only a slight decrease was indicated by the ileum. In one experiment no change was observed on the ear despite the demonstration of a marked increase by the ileum.

III. *Specificity of the Assays.* There is strong evidence that the changes discussed above represent alterations in angiotonin and renin-substrate rather than in nonspecific constituents.

Possible errors in the assay due to changes in adrenalin, hypertensinase, or pH either have been eliminated or have been shown to be insignificant. Parallel changes have been demonstrated with 3 different test objects.

That the substance causing the increased responses in the assays of endogenous angiotonin was actually angiotonin is indicated by the following facts: 1, like angiotonin, this substance was thermostable; 2, it was not inactivated by a concentration of formaldehyde of 40 γ per cc.; 3, it was active in the presence of atropine sulphate in the concentration used; 4, it was not demonstrable in samples from bled bilaterally nephrectomized dogs; 5, it possessed pharmacological actions identical with those of angiotonin on the ileum of the guinea pig, on the blood vessels of the isolated rabbit's ear, and on the blood pressure of the intact dog or cat.

Page (7) recently has reported the presence of a vasoconstrictor substance in dog plasma during shock induced by hemorrhage and other procedures. The substance was found to originate in neither the kidneys nor the adrenals. That

we did not encounter such a substance may be explained perhaps by variations in technique. The following differences may be noted: our routine use of heated rather than unheated samples of plasma; the employment of heparin in place of citrate; the use of calcium-containing Ringer's fluid instead of calcium-free solution. Page does not record experiments in which bilateral nephrectomy and bilateral adrenalectomy had been performed in the same animal. The failure to obtain contraction of the isolated rabbit's ileum is puzzling. In our experiments the ileum of the guinea pig was contracted most consistently by heated plasma from bled intact dogs.

SUMMARY. Dogs anesthetized with morphine and barbital were subjected to hemorrhagic hypotension. Modifications of the procedure of Wiggers and his associates for the production of hemorrhagic shock were employed. Endogenous angiotonin (hypertensin) and renin-substrate (hypertensinogen) were determined semi-quantitatively in plasma by bio-assay on the ileum of the guinea pig, on the perfused rabbit's ear, and on the intact animal. Complication of the assay by endogenous adrenalin was excluded by bilateral adrenalectomy or by inactivation of adrenalin in the samples by formaldehyde. Changes in hypertensinase or in pH did not cause significant error.

Observations concerning renin-substrate. 1. Six of 7 recently nephrectomized dogs, 3 of which also were adrenalectomized, showed progressive increases in renin-substrate during hypotension.

2. A preliminary increase in renin-substrate frequently was observed early in hemorrhage in both intact and adrenalectomized dogs. A similar increase was seen in 2 intact dogs about 20 hours following a single bleeding of 20 to 25 cc. per kgm. of body weight. This phenomenon may constitute a compensatory mechanism in hemorrhage.

3. Following varying periods of hypotension significant reductions in renin-substrate were observed in 10 of 15 intact dogs. The degree and duration of hypotension required to cause depletion of renin-substrate varied markedly in individual animals, and cannot be accurately evaluated at this time. A period of moderate hypotension (e.g., ca. 50 mm. Hg for 90 min.) followed by a period of drastic hypotension (ca. 30 mm. Hg) was required in most animals to produce significant reductions in renin-substrate. A hemorrhage of 20 to 25 cc. per kgm. of body weight about 20 hours previous to the hypotensive period was associated with reductions in renin-substrate following less severe hypotension.

4. Reduction in renin-substrate was observed in 3 of 10 adrenalectomized dogs following hypotension. The data suggest that reduction may occur less readily than in intact dogs.

Observations concerning endogenous angiotonin. 1. Nephrectomized dogs subjected to hypotension failed to show significant rises in angiotonin-like substance.

2. Bled intact dogs consistently showed increases in a substance which contracted the ileum of the guinea pig, elevated blood pressure in dogs and cats, and caused vasoconstriction in the rabbit's ear. This substance is presumed to be angiotonin.

3. A secondary fall in endogenous angiotonin occurred subsequent to depletion of renin-substrate.

CONCLUSIONS

Angiotonin (hypertensin) increases in the plasma of dogs subjected to hemorrhage. Renin-substrate (hypertensinogen), often after a preliminary increase, is reduced if the hypotension is of sufficient intensity and duration. Following exhaustion of renin-substrate there is a secondary fall in angiotonin.

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FURTHER STUDY OF BORON IN THE NUTRITION OF THE RAT¹

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In previous work by Hove, Elvehjem and Hart (1) on the significance of boron in the nutrition of the rat there was some indication that the young of mothers, which were fed a boron low ration, were better able to survive the nursing period when the mothers were fed added amounts of boron. The results obtained by the above authors also indicated that the addition of boron to a basal diet which contained 155 micrograms of boron per kilo did not result in better growth. The data presented in this paper involve (1) the preparation of a ration which would provide a lower boron intake than that previously used, (2) a study of the effect of boron on the growth rate of rats, and (3) an extension of data on the influence of the level of boron on the survival of young rats through the nursing period.

EXPERIMENTAL. *Preparation of Rations.* *Ration A.* Since an extension of the reproduction studies of Hove and co-workers was desired, ration A was prepared exactly as the one used by those workers. It contained 155 micrograms of boron per kilo² and had the following composition:

Acid precipitated casein.....	18
Sucrose.....	69
Salts 3 ³	4
Liver extract powder (1-20).....	4
Corn oil.....	4
Wheat germ oil.....	1
Thiamine.....	2 gamma/gram
Halibut liver oil.....	1 drop/week

Ration B. In an attempt to prepare a ration containing less boron than that already described, the casein, sucrose, salts and liver extract powder of ration A were purified in the following manner: 5 cc. of syrupy phosphoric acid and 200 cc. of methyl alcohol were added to 100 grams of the material to be purified. This suspension was placed on a hot plate and the methyl alcohol evaporated off. The addition of alcohol and the evaporation procedure were repeated 3 to 4 times to drive off boron as the volatile methyl borate.

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² The boron analysis was carried out by a method described by Berger and Truog (2).

³ The salt mixture was composed of NaCl, 167.5 grams; K₂HPO₄, 322.5 grams; CaHPO₄·2H₂O, 75.8 grams; MgSO₄·7H₂O, 102 grams; CaCO₃, 300 grams; Fe-citrate, 27.5 grams; KI, 0.8 gram; MnSO₄, 1.75 grams; ZnCl₂, 0.25 gram; and CuSO₄·5H₂O, 0.30 gram.

Ration B had the same composition as ration A, but had a boron content of 25 micrograms per kilo.

Ration C. Ration C which still contained 25 micrograms of boron per kilo was prepared by substituting synthetic B vitamins for liver extract powder and commercial sucrose for the purified sucrose in ration B. The composition was as follows:

Purified casein.....	18
Purified salts 3.....	3
Sucrose.....	77
Corn oil.....	2
Choline.....	0.1
Thiamine.....	.2 gamma/gram
Pyridoxine.....	.5 gamma/gram
Flavin.....	.2 gamma/gram
Pantothenic acid.....	.10 gamma/gram
Halibut liver oil.....	1 drop/week

Ration D. Ration D which consisted of 55 per cent lard and contained 155 micrograms of boron per kilo had the following composition:

Lard.....	55
Labco casein.....	24
Sucrose.....	15
Purified salts 3.....	6
Choline.....	0.5
Flavin.....	.10 gamma/gram
Thiamine.....	.4 gamma/gram
Pyridoxine.....	.10 gamma/gram
Halibut liver oil.....	1 drop/week

Growth studies. Only two of the rations listed above were satisfactory for these studies, since rations B and C did not support the growth of rats. The animals failed to grow when fed ration C even after the purified casein had been washed with re-distilled water. This point will be discussed later.

Experiment 1. Twenty-six young rats from the reproduction experiments produced by mothers on the low boron basal ration were kept on ration A. Half of them were given 100 micrograms of boron as sodium tetraborate in 100 cc. of drinking water (approximately 40 micrograms per day). The rate of growth was followed by recording the weight of the rats at the beginning of the experiment and at each week for the next six weeks.

Added boron had no effect on the growth rate of these rats as shown in table 1. Since the ration contained 155 micrograms of boron per kilo and the daily food intake of a growing rat was nearer 10 grams than the 5 grams assumed by Hove et al., the rats on the basal diet received a daily intake of approximately 1.5 micrograms of boron.

Experiment 2. The animals in this experiment (40 in number) were transferred together with their mothers from a milk diet to ration D when the young were 14 days old. When the young rats reached the age of 21 days they were divided into

two groups. One received the basal ration D plus 100 micrograms of boron as sodium tetraborate per 100 cc. of drinking water. The other group continued to receive the basal ration. Records of the daily consumption of the diet showed that 4 grams per rat supplied all the requirements and gave fair growth (22-25 grams per week). The partial substitution of the lard for sucrose did not change the boron content from that found in ration A (a high carbohydrate ration). The daily intake of food was lowered and, consequently, the daily boron intake of rats on the high fat basal ration was decreased to a level of about 0.6 microgram per rat.

TABLE 1

Growth of rats on the boron low ration A with and without added boron

Growth in grams per day through a 6 weeks period

MALES		FEMALES	
Low boron	Added boron	Low boron	Added boron
3.0	3.2	2.3	2.8
3.5	3.6	2.3	2.1
3.1	3.9	2.6	2.8
2.4		2.6	2.9
		2.1	2.4
		2.4	2.4
		2.3	2.5
		2.7	2.4
		2.4	2.9
		2.0	
Aver.....3.0	3.6	2.4	2.6

TABLE 2

Growth of rats on the low boron high fat ration D with and without added boron

Growth in grams per day through a 6 weeks period

MALES		FEMALES	
Low boron	Added boron	Low boron	Added boron
3.3	2.9	2.4	2.4
3.7	3.5	2.7	2.4
3.0	3.0	2.9	2.9
3.6	3.0	2.6	3.1
3.6	3.0	2.1	2.7
2.7	3.1	2.4	1.6
2.7	2.6	3.2	2.3
2.7	2.9		2.7
3.1	3.5		2.9
2.9	2.5		2.9
3.6	3.9		
	3.7		
Aver.....3.2	3.1	2.6	2.7

The results of the growth rate of rats during a six weeks' growing period are recorded in table 2. Here again the addition of boron to the low boron basal diet did not result in better growth.

Reproduction studies. Fifty-four 200 gram female rats were fed mineralized milk for one month, then placed on the boron low basal ration A. After 3 weeks they were divided into two groups, one being continued on the basal ration, while the other group received the basal ration plus boron as sodium tetraborate. This was added to their drinking water at a level of 100 micrograms of boron per 100 cc. (approximately 75 micrograms of boron per day). A group of males, which had received the boron low ration for two weeks, was placed with the females.

Pregnant rats were isolated and after the birth of their young, the litters were reduced to 6 or 7 and given to the mothers to nurse until weaning time 21 days later. The number of rats and the per cent weaned were recorded. A total of

97 litters was obtained from 54 females. The females receiving the basal ration plus boron produced 56 litters and gave a reproduction or weaning efficiency of 49.5 per cent. The females receiving only the basal ration produced 41 litters and gave a weaning efficiency of 50 per cent. The complete data are given in table 3.

DISCUSSION. *Growth studies.* According to the early work of Hove et al. (1) a ration carrying a daily intake of 1.55 micrograms of boron per rat seemed to satisfy the boron requirement if this element were needed at all. A ration lower in boron content was, therefore, essential if further information on this problem were to be obtained.

TABLE 3

The reproduction efficiency of female rats on ration A with and without added boron

NO. OF FEMALES	NO. OF LITTERS	NO. OF PUPS	AVER. NO. OF PUPS/LITTER	NO. OF PUPS WEANED	PER CENT WEANED
Added boron					
5	16	115 (101)*	7.2	67	66.3
6	10	72 (58)	7.2	24	41.4
6	7	60 (41)	8.6	20	48.8
5	9	63 (47)	7.2	8	17.0
5	14	145 (86)	10.5	46	53.4
Total.....27	56	455 (333)	8.0	165	49.5
Low boron basal ration					
5	9	65 (58)	7.2	37	63
6	4	22 (22)	5.5	2	9
6	11	92 (66)	8.4	34	51.5
5	6	42 (31)	6.6	15	48.5
5	11	124 (67)	11.5	34	50.7
Total.....27	41	345 (244)	7.8	122	50.0

* The number in parentheses represents the number of young left with the mothers for nursing.

Since in the determination of boron in complex organic materials, the boron was separated from the ash mixture by evaporation as the methyl borate, it was thought feasible to purify casein, sucrose and salts by this procedure. Analysis of the ration after purification showed a boron content of 25 micrograms per kilo (155 micrograms per kilo before purification). However, when this ration was fed the rats failed to grow normally and, in fact, many lost weight. It was first believed that the cause was the formation of loose bonds such as hydrogen bonds between the methyl alcohol and sucrose or casein. The nature of the compounds could easily lead to such linkages. To test whether or not the hydrogen bond was the only type of linkage involved, the casein of the diet was purified by the

methyl alcohol method and then washed with water. Washing should break the hydrogen bond and hence restore the original growth properties of the ration. After this procedure was carried out, however, the rats on the ration still did not grow normally. This indicated that either a denaturation of the protein had taken place or esters of methyl alcohol, which could subsequently be hydrolyzed by the animal, had been formed. It would seem very likely that both denaturation and ester formation resulted.

Since animals can satisfy their caloric requirements with a lower intake of a high fat ration than with a high carbohydrate ration, a diet, consisting of 55 per cent lard and containing an amount of boron (155 micrograms per kilo) equal to that in the high carbohydrate ration, was finally used for growth studies. The food intake on this type of ration was 4 grams per day and the boron intake was 0.6 microgram per rat per day. Even with this low boron intake no differences in growth rate were obtained between rats on the basal diet plus boron and those on the basal ration alone. Again the conclusion must be limited since the basal ration still was not absolutely free of boron. Perhaps this problem can be definitely solved when rations made up of purely synthetic materials are devised. The use of synthetic amino acids, essential fatty acids, synthetic vitamins, pure salts and cerelese would help very much in the establishment of the nutritional significance of boron as well as other elements which have not been heretofore used in experiments on nutrition.

Reproduction studies. Although the weaning efficiency was poor in both groups of the boron reproduction experiments there was no significant difference between the basal group and the group receiving additional boron. This is in contrast to the indications obtained by Hove and co-workers (1) in a preliminary study.

It is important to note that 200 gram rats on a ration such as used in these reproduction studies contain only 2 micrograms of boron per rat. This in itself would lead one to doubt the essential nature of boron. If boron is needed for reproduction, the requirement is satisfied by 155 micrograms per kilo of ration.

To carry out a more critical study of this problem a ration having a lower boron content must be found as well as one that would give better reproduction.

SUMMARY

1. A diet purified by driving off boron as volatile methyl borate showed, on analysis, a boron content of 25 micrograms per kilo. But this ration did not support growth even when boron was added. Washing the purified casein with water did not restore the growth properties of the ration. The casein had either been denatured or it had been esterified with methyl alcohol.

2. A high fat ration containing 55 per cent of lard had a boron content equal to that of a high carbohydrate ration. The growth rate of rats on this ration was fair (22-25 grams per week). Consumption studies showed that the intake of the high fat ration was about one-half the intake of the high carbohydrate ration, thus giving a boron intake of 0.6 microgram per rat per day. Additions of boron

to this low boron ration did not improve the rate of growth. It is evident that if boron is needed by the rat for normal growth then 0.6 microgram per rat per day satisfies that requirement.

3. The addition of boron to a basal ration containing 155 micrograms of boron per kilo did not improve the ability of rats to nurse their young through the weaning period.

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CARDIAC OUTPUT AND TOTAL PERIPHERAL RESISTANCE MEASUREMENTS IN EXPERIMENTAL DOGS¹

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The degree of constancy of cardiac output which is maintained in anesthetized experimental dogs has not been established with sufficient certainty to permit a satisfactory quantitative evaluation of subsequent alterations induced by experimental procedures. Estimates of total peripheral resistance (TPR), which require accurate measurement of cardiac output and mean arterial blood pressure, are scarce and have usually been obtained under extremely unnatural conditions.

This communication describes important refinements of a procedure for measuring cardiac output initially introduced by G. N. Stewart (1, 2). This "modified Stewart method" is at least as accurate as the Fick method and possesses the advantage of permitting *frequent* successive estimates and comparisons of cardiac output and hence total peripheral resistance during protracted experimental periods. A statistical analysis of 145 control determinations on 42 dogs has yielded valuable information which is an essential prerequisite to the study of the changes which lead to and occur during circulatory failure or shock in the intact dog.

The method for determining cardiac output as originally proposed by G. N. Stewart (1, 2) begins with the infusion of NaCl solution directly into the right or left heart. After mixing with the blood in the heart chambers, the blood-NaCl mixture is transported to outlying systemic arteries from one of which (femoral) a sample is withdrawn. A control blood sample, collected prior to the infusion, is titrated with the same NaCl solution until its conductivity equals that of the test (blood-NaCl) sample. Thus the dilution of the infused NaCl by the volume of blood entering the heart per unit of time is determined. Having ascertained this dilution factor m , and measured the rate of saline infusion, the volume of blood entering the heart per minute and therefore the cardiac output can be rapidly calculated from the relationship $V = \frac{q \times 60}{mt}$, in which

V is the cardiac output in ml./minute, $\frac{q}{t}$ the rate of uniform NaCl injection into the right heart, and m the quantity of salt in each milliliter of the blood-NaCl mixture. This volume of salt solution, even when injected *repeatedly*, is rapidly diluted by recirculation and excretion by the kidneys. Hence, it does not interfere with repeated determinations as do dyes and other substitutes. For

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additional consideration of theoretical and experimental aspects of the method the articles of Stewart (1, 2) and of Hamilton and his co-workers (3-6) should be consulted. From a technical standpoint, infusion into the right atrium is preferable; it is easier, less disturbing to the natural circulation and assures a more complete mixing of the salt solution and blood during its initial circulation.

Inasmuch as the successive values for cardiac output obtained and reported by Stewart (2) were quite variable, the procedure has been regarded as unsatisfactory and has not been utilized in the intervening years. Stewart himself

TABLE 1

AUTHOR	WT. RANGE	ANESTHESIA	METHOD EMPLOYED	NO. OF DOGS	NO. OF TESTS	CIRCULATORY INDEX	
						Mean	S.D.
G. N. Stewart (2).....	kgm. 7-33	Morphine and ACE mixture	Stewart	7	46	3.90	±0.89
G. N. Stewart (2).....	5-28	Morphine and ether	Stewart	6	33	4.27	±0.70
T. Harrison et al. (7)....	5-13	Morphine	Fick	20	30	2.98	±0.65
J. W. Moore et al. (8)...	15-22	Morphine	Fick dye inject.	3	3	3.68	
				3	3	3.63	
J. W. Moore et al. (8)...	15-22	Na barbital	Fick dye inject.	3	3	3.13	
				3	3	2.84	
W. F. Hamilton (9).....	8-16	Morphine	Dye inject.	6	6	4.96	
Tappan et al. (10).....	8-18	Morphine	Fick	7	13	2.63	±0.70
Tappan et al. (10).....	8-11	Urethane	Fick	7	7	3.85	±1.72
T. Harrison et al. (11)...	9-14	Unanesthetized	Fick	9	10	4.12	±0.76
E. K. Marshall, Jr. (12).	12-20	Unanesthetized	Fick	5	91	3.09	±0.83
A. Cohn et al. (13).....	10-20	Unanesthetized	Fick	7	7	6.41	±2.71
H. J. Stewart et al. (14).	10-20	Unanesthetized	Fick	5	6	2.58	±0.72
H. Rasmussen (15).....	7-12	Unanesthetized	Fick	5	18	2.75	±0.59
H. C. Wiggers (this paper)	10-31	Morphine and Na barbital chloralose or pentobarbital	Modified Stewart	42	145	2.87	±0.44

attributed this variability partly to actual fluctuations of cardiac output ensuing from the employment of volatile anesthetics and partly to a lack of any attempt to maintain stable control conditions. Preliminary investigations convinced the author that certain technical procedures employed by Stewart and the apparatus available at that time could not have been expected to yield more consistent results. It is interesting, however, as can be seen in table 1, that even greater discrepancies in "control" determinations of cardiac output have been reported by investigators who have employed the generally accepted Fick method. After conducting these trial experiments, however, it seemed

probable that with certain technical refinements and with the aid of improved electrical apparatus which modern development affords, the method might prove satisfactory in a quantitative way and at the same time permit many more determinations over a given interval than other methods which are applicable to intact dogs.

TECHNICAL PROCEDURE. In order to obtain consistent quantitative values by this method certain technical details must be meticulously executed. 1. The salt solution must flow into the right heart at a constant rate, not at a decelerating rate as from an open burette, employed by Stewart. To insure the latter, salt solution in a Schellbach burette, graduated to 0.02 ml., was subjected to a constant pressure (circa, 160 mm. Hg) during the injection interval by means of a reducing valve interposed in a laboratory air line. The burette emptied through the resistance of a 6 cm. segment of capillary glass tubing which was connected by a short section of rubber tubing with the sound, previously introduced via a jugular vein into the right atrium with its tip near the A-V valve. Occasionally, it was introduced into the right ventricle without any apparent difference in the results. The onset and completion of each infusion were signalled on a kymograph with the mean arterial blood pressure and also on a photokymograph recording central arterial pressure pulses and occasionally an electrocardiogram. The rubber connecting tube was fixed beneath a signal key, adapted to compress the tubing when the key was pressed. The opening and closing of this key (a) initiated and checked the infusion of saline, (b) signalled the event on records and in addition (c) actuated a chronoscope, reading accurately to 0.01 second. With this arrangement, q and t could be determined immediately by reading the burette and chronoscope respectively, as well as calculated from the records.

2. The concentration and rate of injection of the NaCl solution must be adjusted so that it produces a detectable difference in the conductivity of the blood passing through the femoral artery without significantly altering the cardiac output either through changes in cardiac rate, rhythm or filling. In preliminary experiments, such temporary disturbances occurred frequently when 5 per cent NaCl solution was used and generally with a 10 per cent solution. Obviously, if this happens, cardiac output determinations cannot be considered as those of the dog under natural conditions. After testing numerous combinations of salt concentration and rate of injection, it was found that the intra-cardiac injection of about 10 ml. of 3 per cent NaCl solution at a uniform rate of 0.75 ml./sec. is sufficient to produce a detectable change in the conductivity of blood without altering the cardiac rhythm, the arterial blood pressure or the pulse pressure.

A typical optical record (fig. 1) obtained during the injection of 3 per cent NaCl solution (A-C) and during the withdrawal of a blood-NaCl sample (D-E) reveals that neither the venous pressure values nor the magnitude or contour of the arterial pressure curves registered by sensitive artificial membrane manometers are significantly influenced by either procedure. In the record reproduced, the heart rate remained constant at 140 beats per minute. Since the signal

deflection at B represents the onset of imbalance in the bridge circuit across the femoral blood stream, due to the initial arrival of the injected salt, the right atrial-femoral artery *circulation time* is represented by the interval A-B.

To further check the effects of 3 per cent NaCl infusion on stroke volume, a number of tests were conducted on dogs with an open thorax while cardiometric records of the heart beat were being resistered. The latter revealed that the injection procedure described above induces no measurable alteration in the stroke volume even when the heart rate was slightly reduced. Such tests indicate that at this rate of injection the cardiac output is definitely not increased and that determinations made are those natural to the dog studied. Apparently this was not regularly achieved by the technique as employed by Stewart.

3. The time of arrival of the uniform blood-NaCl mixture at a point in the femoral artery must be clearly indicated by imbalance of a Wheatstone bridge. This can only be accomplished satisfactorily by maintaining a constant distance between electrodes across the blood stream and by the employment of an electrical detecting instrument of adequate sensitivity. When electrodes were

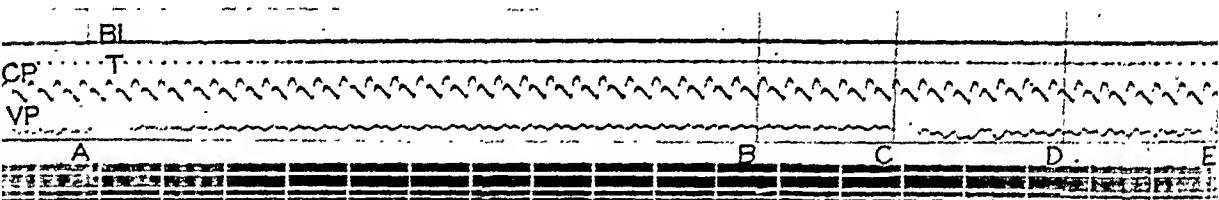


Fig. 1. Typical optical record demonstrating the negligible effects of intracardiac infusion of 3 per cent NaCl solution (A-C) and withdrawal of blood-NaCl sample (D-E) on central arterial (CP) and intra-atrial pressures (VP). Time (T) in 0.02 second. BL = baseline. A-B interval = right atrial-femoral artery circulation time.

applied externally to a femoral artery, opposite to the one used for collecting blood samples, as Stewart suggested, apparent variations in conductivity were frequently recorded. These were due to shifts of electrode contacts on pulsating vessels and to occasional slight movements of the extremities. Such apparent variations occurred especially when significant changes in the blood pressure between tests caused either collapse or expansion of the femoral artery. Elimination of these sources of error, the maintenance of continuous flow through the artery from which samples were collected and the preservation of one femoral artery for other purposes were all accomplished by the insertion into a femoral artery of a specially designed "detection-sampling cannula" (fig. 2). Its construction in four easily assembled units facilitates cleaning and insertion into the artery. The major portions of the cannula (units *T*, *T'* and *E*) are constructed of lucite in order to prevent shunting of the minute bridge current away from rather than across the electrodes. Unit *E* is essentially a miniature conductivity cell in which excellent contact of blood is assured with the pair of platinum electrodes which encircle the lumen and are always 8 mm. apart. By means of exteriorized binding posts, these electrodes are connected with a

sensitive 1000 cycle A.C. conductivity bridge. As an additional precaution against short-circuiting of the bridge current, a sheet of rubber is placed between the lucite cannula and the exposed tissues of the leg. Circuit balance in the latter immediately prior to the infusion is indicated by maximal opening of the dark segment of a cathode-ray visual null indicator. Introduction of a variable condenser across the fixed resistance of the bridge improves the operation of this indicator. A shift to a position of constant minimal opening indicates passage of the uniform blood-NaCl mixture across the electrodes. During this period, a 5 ml. sample of the mixture is rapidly drawn into a syringe, fitted by sleeve attachment to the side tube of the brass sampling unit *S*. To withdraw blood,

FIG. 4

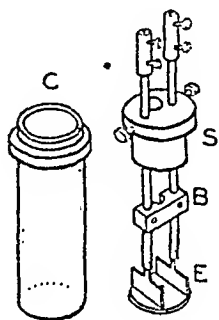


FIG. 3

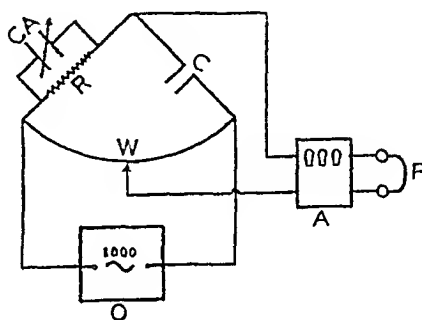


FIG. 2

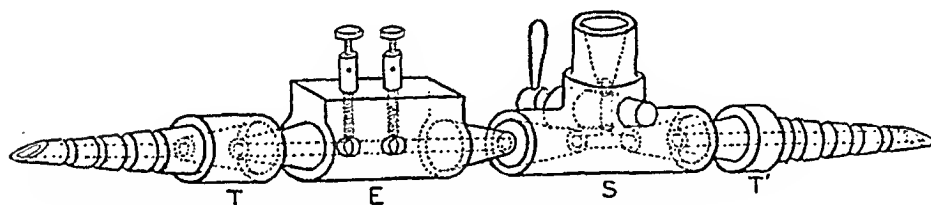


Fig. 2. "Detection-Sampling Cannula." Described in text.

Fig. 3. Schematic representation of Wheatstone A-C bridge circuit employed for electro-titration and conductivity measurements. Discussed in text.

Fig. 4. Special conductivity cell for measuring conductivity of small quantities of non-homogeneous solutions such as blood, with special device for agitating fluid contents. Discussion in text.

the miniature stopcock *S* is turned to the open position, as indicated in the drawing.

4. The arterial sample must be drawn only during the passage of the uniform blood-NaCl mixture. If taken before or after, sizable errors in the cardiac output determinations result. A risk of error is incurred in any procedure in which withdrawal is made from a centrally cannulated artery (as per Stewart) through which blood is not continuously flowing. In this "detection sampling unit" there is no dead space for collection and stagnation of blood which is likely to be included in a sample, since the stopcock is constructed to open flush with the lumen of the vessel. The lumen of the stopcock is filled with an anticoagulant solution, liquaemin,³ (0.1 ml.) prior to each determination.

³ We are indebted to Roche Organon, Inc., Nutley, N. J., for the liquaemin used in these experiments.

5. The electro-titration of a previously obtained control blood sample with 3 per cent NaCl solution sufficient to equalize its conductivity with that of the test sample requires, in addition to the technical equipment essential for satisfactory conductivity measurements of homogeneous solutions, a proper means of making adjustments for sedimentation of red blood corpuscles. Their removal by centrifugalization and the conduction of all tests on plasma samples introduces other obvious difficulties and defeats the time-saving element of this method.

Commercial conductivity cells were found inapplicable for this purpose because they require too large quantities of blood and provide no suitable means for agitating this non-homogeneous medium. It was found necessary to adopt an arbitrary standard procedure for agitating the blood sample immediately (30 sec.) prior to measuring its conductivity. To prevent the influence of sedimentation a conductivity cell was constructed with these provisions in mind as shown in figure 4. The container unit *C* is made of lucite. The electrode unit consists of a pair of platinum black electrodes, *E*, firmly anchored at a distance of 1 cm. by a circular lucite disk. The insulated metal rods which extend from the electrodes to the exterior binding posts are maintained at a fixed distance by a lucite brace *B* and again by screw adjustments as they traverse small apertures in the lucite stopper, *S*. Thus, a consistent cell constant was maintained at all times. The small circular aperture in the disk between the pair of electrodes provides a means of agitating the blood by gently raising and lowering the electrode unit within its container. The electrodes are just immersed by 4 ml. of fluid (blood). The eccentric perforation in the lucite stopper, *S*, provides a means of adding salt solution to the control blood sample.

Titration of the control blood sample with 3 per cent salt solution must obviously be conducted with the utmost precision since a minute error in this procedure leads to one of considerable magnitude in the final computation. In order to deliver a quantity as small as 0.002 ml. of saline at any time, a fine paraffined glass capillary tip was added to a calibrated pipette, graduated to be read accurately to 0.002 ml. Delivery was achieved by a precision micrometer screw which compressed a rubber chamber attached to the top of the burette. The Wheatstone bridge circuit is schematically represented in figure 3. At the beginning of an experiment, a fixed resistance, *R*, is introduced which approximately balances that of the blood stream, generally of the order of 70-100 ohms. The 1000 C.P.S. tone in the earphones was provided by an oscillator, *O*. By employing this frequency, a tone in the most sensitive range of audibility was obtained and at the same time development of a polarization E.M.F. at the platinum black electrodes of the conductivity cells was eliminated, as frequent checks at higher frequency currents revealed. Further enhancement of the accuracy of conductivity measurements was accomplished (a) by annulling electrostatic capacitance within the cell by the introduction of a variable condenser, *CA*, across the fixed resistance terminals, and (b) by inserting a 3-stage audio-amplifier, *A*, in the earphone circuit, *P*, to permit desired variation in the in-

tensity of the tone during the conductivity measurements. As a result, silence in the phones was obtainable at the null point in all determinations and the null point was limited to less than 0.5 unit on the slide-wire scale, W , which was graduated in 2000 units. However, although conductivity can be measured very precisely, the accuracy of titrating to match conductivities of two blood samples is limited by the characteristics of the titration apparatus. The error involved in the titration procedure may amount to 3 to 5 per cent under unusual conditions. Each determination was made with the cell immersed in a temperature bath constant to 0.001°C . between any two measurements. In general, the bath and hence sample temperatures were at 35°C .

At this point a concise summary of the sequential procedure employed in these cardiac output studies appears expedient. 1. A 5 ml. control blood sample is collected from a femoral artery via the "detection-sampling cannula." 2. An A-C Wheatstone bridge circuit is balanced across the femoral blood stream. 3. A measured quantity (10 ml.) of 3 per cent saline solution is infused at a uniform rate (ca. 0.75 ml./sec.) into the right atrium or ventricle. 4. While a uniform blood-NaCl mixture passes the femoral electrodes, as indicated by a constant imbalance of the Wheatstone bridge, a 5 ml. sample of the blood-NaCl mixture is withdrawn into a syringe with a constant though minute quantity of liquaemin. 5. The amount of salt (m) in each milliliter of the latter sample is determined by matching its conductivity with that of the control sample to which 3 per cent NaCl has been meticulously added. 6. Having ascertained m , t and q values, the minute cardiac output is computed.

The advantages of this method over others are: (a) It eliminates many time-consuming analyses and computations, (b) it provides the investigator with a value for the cardiac output within 15 minutes of the time that the determination is begun, (c) repetitive determinations can be conducted at 5 minute intervals, and (d) it requires only minute quantities of blood, which may be reinfused later if desired. With every 10 ml. of blood withdrawn the animal receives 10 ml. of fluid (3 per cent NaCl injection).

Criteria of the reliability of the method. The initial portion of this investigation was directed to examine the reliability of the method. The criteria of reliability that may be used are; (a) the soundness of the principles upon which the method is conceived and the probability of their being satisfied in practice with the technical procedures involved, (b) the ability to obtain quantitative values which agree reasonably with those obtained by some known satisfactory method, if such exists, and (c) the ability of the method to reproduce results of reasonable magnitude in successive determinations.

Although a comparison of values obtained by the *modified Stewart* and the Fick methods was made in this investigation (see table 4), there is reason to believe that the degree of reliability generally accredited to the Fick method when applied to dogs is based largely upon its sound theoretical foundation rather than upon demonstrated facts. For this reason, greater emphasis is placed upon the attainment of reasonable as well as consistent results in consecutive deter-

minations. However, such consistency obviously cannot be expected if cardiac output varies during the period of determinations. A chemist would not expect consistent results if his test solutions changed from time to time.

Although this method, with perhaps some slight modifications, appears applicable for a study of cardiac output in the well-trained unanesthetized dog, there are several reasons why these experiments were conducted on animals during prolonged periods of even anesthesia. Experience has shown that whereas some fluctuations in the circulatory state may occur, they are less frequent and less extensive than those commonly encountered in the unanesthetized animal. This fact assumes major importance when consistency in consecutive cardiac output determinations is sought. The slightest emotional excitement or discomfort induces respiratory, heart rate, muscle tonus and positional changes which may suffice to alter the cardiac output significantly.

These studies were conducted on 42 dogs whose weight varied from 10 to 31 kgm. Following a sedative dose of morphine (1-2 cc. of 20 per cent solution), 33 dogs received sodium barbital (175 mgm./kgm.), 6 received chloralosane (75-80 mgm./kgm.) and the remaining 3 received sodium pentobarbital (125 mgm./kgm.). Cardiac output determinations were begun circa 3 hours after administration of the anesthesia, thus allowing ample time for stabilization of the circulation. Operative procedures entailed (a) insertion of the "detection-sampling cannula" into a femoral artery; (b) arrangement for kymographic registration of mean arterial blood pressure from the femoral artery; (c) introduction of a cardiac sound into the right atrium via the right jugular vein and (4) connections for optical registration of central arterial pressure curves by a high frequency membrane manometer. While not absolutely necessary, an anticoagulant, liquaemin, was administered intravenously to preclude clotting of blood within the "detection-sampling cannula."

RESULTS. Some of the details of results obtained from individual dogs are incorporated in tables 2 and 3, arranged in accordance with the weights of the animals. The number of determinations made during stabilized periods of anesthesia and the duration of the experimental periods are indicated.

In order to reduce all values to an equivalent standard, mean cardiac outputs in the various animals and their standard deviations are reported on a surface area basis (*circulatory index*);⁴ but the actual stroke volumes are given. In most animals some variation in heart rate occurred between consecutive measurements indicating that complete stability is not always attainable even in anesthetized animals. Moreover, in some, the mean arterial pressure remained stable, while in others it also varied considerably.

Since it was apparent from analysis of the results that consecutive cardiac output determinations vary considerably with changes in the arterial pressure and but little with fluctuations in heart rate, these animals were grouped on the basis of the stability or instability of their mean arterial pressures. The former,

⁴ The term "circulatory index," suggested by Yandell Henderson (21) seems preferable to the term "cardiac index" proposed by Grollman (16), which has rather uncertain implications.

incorporated in table 2, afford material for checking the consistency of successive readings and yield information regarding cardiac output and total peripheral resistance under stabilized conditions, whereas the latter, compiled in table 3 allow deductions with regard to the extremes in variability of cardiac output that may be expected in experimental animals which have been subjected only to inconsequential operative procedures.

(A) *The reliability of results.* In the 20 dogs shown in table 2, in which mean arterial pressure fluctuations were slight (i.e., 7 mm. Hg; S.D. ± 5), the differ-

TABLE 2

EXP.	WT.	EXPERI- MENTAL PERIOD	NO. OF TESTS	MEAN CARDIAC OUTPUT AND S.D.	RANGE OF HEART RATE	RANGE OF MEAN B.P.	AVERAGE STROKE VOL.	AV. TOTAL PERIPHERAL RESISTANCE
	kgm.	min.		l./sq.m./min.	per min.	mm. Hg	ml.	abs. units†
1	31.0	50	3	2.87 \pm 0.03	160-180	143-153	18.13	3700
2	26.5	35	3	2.86 \pm 0.01	155-163	145	18.05	4100
3†	26.5	50	3	2.50 \pm 0.10	140-188	165-175	14.78	5600
4	23.0	60	4	3.08 \pm 0.04	190-195	155-162	14.50	4600
5	21.5	95	3	2.92 \pm 0.03	190-200	133-138	13.07	4300
6	21.0	25	2	3.37 \pm 0.06	155-170	161-162	17.72	4500
7	21.0	45	3	2.00 \pm 0.08	245-250	148-155	6.89	7100
8	20.5	50	3	1.99 \pm 0.09	95-165	125	14.33	6000
9	20.5	50	3	3.50 \pm 0.06	150-158	125-127	19.27	3500
10	20.0	155	4	2.78 \pm 0.05	140-158	98-103	16.25	3500
11	19.0	150	4	3.02 \pm 0.08	137-150	160-180	16.72	5400
12*	18.5	60	2	2.78 \pm 0.02	200-204	105-120	10.79	4200
13*	17.0	40	2	3.46 \pm 0.00	160-166	125-130	15.72	4000
14†	17.0	65	3	2.73 \pm 0.07	130-150	110-120	14.83	4600
15	16.5	95	3	2.88 \pm 0.07	210-230	125-135	9.42	5100
16	16.5	45	3	3.37 \pm 0.03	130-160	110-115	17.18	3600
17	16.2	45	2	2.43 \pm 0.05	172-178	92-95	10.02	4300
18*	16.0	65	3	2.42 \pm 0.07	160-185	78-90	9.94	3800
19	12.5	100	4	2.15 \pm 0.07	165-175	80-88	7.70	6100
20	10.0	45	3	2.35 \pm 0.05	146-164	143-147	7.95	9400
Average values.....				2.77 \pm 0.05	17; S.D. 16	7; S.D. 5		

* Dogs under nembutal.

† Dogs under chloralosane.

‡ See text for definition and method of calculating TPR.

ences in consecutive determinations on the same animal were very small (S.D. 0 to ± 0.10). The mean *circulatory index* was 2.77 and the average deviation of the individual variations was only ± 0.05 . In the remaining 22 dogs listed in table 3, in which arterial pressure fluctuated more (18 mm. Hg; S.D. ± 13), the variability of successive determinations was somewhat greater but not excessively so (S.D. ± 0.10 to ± 0.39). The mean *circulatory index* in this group was 2.94 and the average of the individual deviations was 0.19. By combining the 145 determinations in 42 dogs, a mean *circulatory index* of 2.87 with a standard deviation of ± 0.44 was obtained. These values may be advantageously

compared, as in table 1, with results reported by others using various methods on both anesthetized and unanesthetized dogs. It may be seen at a glance that existing values reported for the *circulatory index* of dogs not only show extreme variability but that the average S.D. from the mean obtained from all animals in this investigation is the lowest of the entire group. Thus, such values appear more favorable than those obtained in most animals in which the Fick principle was employed. On any statistical basis the conclusion seems warranted

TABLE 3

EXP.	WT.	EXPERI- MENTAL PERIOD	NO. OF TESTS	MEAN CARDIAC OUTPUT AND S.D.	RANGE OF HEART RATE	RANGE OF MEAN B.P.	AVERAGE STROKE VOL.	AV. TOTAL PERIPHERAL RESISTANCE
	kgm.	min.		l./sq.m./min.	per min.	mm. Hg	ml.	abs. units†
21	26.0	125	4	2.78 \pm 0.11	200-210	155-174	13.54	4800
22	23.5	115	5	2.95 \pm 0.12	200-225	120-142	12.53	3900
23	23.5	185	4	2.98 \pm 0.11	135-161	125-135	17.94	3400
24	23.0	100	5	2.71 \pm 0.14	122-137	125-165	18.68	4600
25	22.0	85	3	2.88 \pm 0.12	180-200	122-135	13.27	4100
26	22.0	100	4	3.37 \pm 0.22	163-182	110-120	16.86	3100
27	20.0	230	7	2.69 \pm 0.27	118-180	110-125	15.84	4300
28*	19.5	40	3	3.56 \pm 0.13	150-158	148-158	18.70	4200
29	19.0	60	3	3.05 \pm 0.28	210	145-150	11.30	5000
30	18.5	70	4	3.24 \pm 0.27	193-224	130-140	11.95	4300
31	18.0	55	3	2.66 \pm 0.12	118-140	122-160	16.18	5000
32	18.0	145	6	2.71 \pm 0.39	184-205	120-140	10.45	5300
33	18.0	75	4	2.77 \pm 0.30	206-210	110-120	10.26	4100
34*	17.5	60	3	2.79 \pm 0.18	190-214	125-140	10.40	5000
35*	17.0	120	5	4.18 \pm 0.23	204-230	125-135	14.62	3400
36	17.0	40	3	1.97 \pm 0.24	210-222	120-130	6.70	6900
37	16.0	50	3	3.01 \pm 0.17	108-180	150-160	15.02	5700
38*	16.0	60	3	2.41 \pm 0.16	165-210	145-160	8.96	6600
39	14.0	60	3	3.24 \pm 0.25	200-203	94-155	10.43	4600
40	13.5	12	2	2.88 \pm 0.10	50-57	130-152	34.20	6100
41	13.0	105	3	2.75 \pm 0.15	205-216	118-145	8.18	6600
42	12.5	210	5	3.07 \pm 0.17	90-98	109-115	19.47	4900
Average values.....				2.84 \pm 0.19	21; S.D. 18	18; S.D. 13		

* Dogs under chloralose.

† See text for definition and method of calculating TPR.

that the *modified Stewart method* certainly yields as accurate and consistent results as any other method.

As a further check, comparative determinations by the *modified Stewart* and Fick methods were made as nearly simultaneously as possible in 5 dogs. Arterial blood samples for Fick analysis were collected from a femoral artery. Venous samples were withdrawn via the cardiac sound from the right ventricle, precautions having been previously taken to wash out all of the 3 per cent NaCl from the sound. The procedure had no measurable effect upon the blood pressure or heart rate. The O₂ content of the arterial and venous samples was de-

terminated by the Van Slyke manometric method.⁵ The O₂ consumption was measured continuously in these animals both during and between all determinations by a "blower type" metabolism machine connected to a tracheal cannula. A five minute interval supervened between the two comparative determinations in each instance. The results are presented in table 4. The circulatory state between these tests did not remain stable for long periods of time. In fact, it seemed desirable to observe how well these determinations agreed under distinctly variable conditions. Hence the cardiac output measurements in the two animals indicated by asterisks in table 4 were conducted under abnormal conditions incurred by extensive hemorrhages. It is evident that the minute volume outputs were as comparable as could be expected and that this holds for animals in the hypotensive as well as in the normotensive state. On the whole, the car-

TABLE 4

EXP. NUMBER	DOG WT.	MEAN B.P.	CARDIAC OUTPUT	
			Modified Stewart	Fick
	kgm.	mm. of Hg	ml./min.	ml./min.
23	23.5	130	2660	2220
		135	2760	2520
27	20.0	135	2540	2520
		114	2280	2280
		105	1955	2040
30	18.5	140	2490	2600
43*	18.5	62	876	778
		50	750	644
34*	17.5	130	2040	1750
		115	1580	1450
		80	1010	945

* Animals in abnormal condition as a result of induced hemorrhages.

diac outputs obtained by the *modified Stewart technique* were slightly larger, although in several instances they were identical or slightly greater in the Fick evaluation.

(B) *Normal circulatory index for dogs. 1. Constancy in different dogs* On certain occasions it is convenient for the investigator to have available the cardiac output expressed in milliliters or liters per minute. It has become customary, however, to express minute cardiac output on a body surface area basis (liters/sq. meter/min.) when comparisons in different animals are to be made.

It is expedient to consider the constancy which can be anticipated for cardiac outputs in a random group of mongrel laboratory dogs and interesting to com-

⁵ The author is indebted to Dr. J. Waide Price and Dr. Margaret Daus of the Department of Medicine who performed the gas analyses.

pare this constancy with that obtained by Grollman's extensive study in man where control conditions could be rigidly maintained. The remarkable constancy obtained by the latter investigator in human subjects is shown in the small deviation (± 0.30) from the mean *circulatory index*. Grollman also observed that certain apparently trivial events such as 1, ingestion of foods or liquids; 2, slight psychic disturbances; 3 failure to obtain complete relaxation from previous physical activity; 4, insufficient or disturbed rest during the preceding night, and 5, slight indisposition or malaise ascertainable only by subjective questioning, to name a few, introduce marked variations in the *circulatory index*. Most of these events are not adequately controllable in dogs. The variable response of individual dogs to anesthesia must be added to the list. A further hint as to the degree of constancy to be expected in laboratory dogs is furnished by Hamilton's (17) study of control blood pressures in 215 mongrel dogs. He reports fluctuations in blood pressure which indicate that states of hypotension as well as hypertension are not infrequently encountered, as well as seasonal variations and senile deterioration of the heart.

The mean *circulatory index* in different dogs as reported by various investigators has a very wide range (table 1). Undoubtedly, these wide discrepancies are in large part attributable to (a) technical errors in measurement; (b) unsatisfactory control conditions, and (c) the failure of many of these investigators to obtain more than a solitary control determination. In considering these findings, it seems reasonable to anticipate that if the dogs are in good physical condition and the circulation remains fairly stable, most determinations of the *circulatory index* will fall within a range of 2.50 to 3.30. Undoubtedly, many will fall in a more restricted range, say 2.70 to 3.00. The determination of these ranges is speculatively based upon determinations of reasonable magnitude reported by investigators whose data showed no greater standard deviation from the mean than 0.83 liter/sq.m./min. There can be no question, therefore, that the constancy of this index in man cannot be compared with such inconstant values as have been reported for the dog. As shown in table 1, the greatest degree of constancy of the index that has been obtained in dogs is found in the results reported in this paper; and they reveal a standard deviation from the mean of 0.44 liter/sq.m./min.

(2) *Species comparison of the circulatory index*. In 1929 Marshall (18) compared the output index obtained by various investigators in man, dogs, goats, rabbits and horses. It is not clear why Marshall, who in the same article recognized and praised the importance of Grollman's constant basal output index values of 2.2 ± 0.30 for man, employed the comparatively high value of 2.62 liters/sq.m./min. for man in comparing it with the value 2.86 for dogs. Obviously, if Grollman's generally accepted values are used, the *circulatory index* of man and dog are not of the same order of magnitude. The range of the mean index in the above 5 species varied from 1.69 in the rabbit to 3.07 in the goat, since he excluded the values for the horse (5.81) as technically erroneous. Marshall concluded that the "values per square meter of body surface area were of the same order of magnitude." In view of the fact that the value in the rabbit

was approximately one-half that in the goat, such a conclusion seems totally unwarranted. If the values he presents are representative of the actual *circulatory index* in these species, it again seems futile to seek any degree of equivalency in the index of man and dog.

(3) *Cardiac output and animal size.* The arbitrary classification of the 42 dogs into medium sized, large-sized and extra-large sized dogs was arranged to retain a satisfactory number of animals in each category (table 5). Since the constancy of determinations in these 42 animals was quite good, it was not surprising to find the *circulatory index* for all three groups to be of the same order of magnitude. However, the minute volume decreases as expected when the body weight decreases. Likewise, the stroke volume is less in smaller animals. The variations might have been greater in the three groups had the heart rate not been slower in the smaller animals.

(4) *Need for repeated control determinations.* Since the apparent range of normalcy for control cardiac outputs is rather wide in mongrel laboratory animals, it seems necessary to re-emphasize the need for repetition of control de-

TABLE 5

SIZE OF DOG	NO. OF DOGS	NO. OF TESTS	CARDIAC OUTPUT (ML./MIN.)		AVERAGE				
			Av.	S.D.	Circulatory index	T.P.R. (A.U.)	Stroke volume	Heart rate	Circulation time*
									sec.
Extra-large, 21-31 kgm.....	13	46	2660 \pm 330		2.87	4230	15.8	181	11.5
Large 17-20.9 kgm.....	17	66	2240 \pm 490		2.93	4630	13.8	174	10.2
Medium 10-16.9 kgm.....	12	37	1820 \pm 340		2.75	5570	11.3	161	9.5

* Circulation time in seconds from right atrium to femoral artery.

terminations until constant results are obtained prior to the onset of experimental procedures. The literature from which table 1 was compiled contained many reports wherein the investigators were apparently satisfied with a single control determination of cardiac output. Hence, the validity of such values is difficult to establish. The value of satisfactory control determinations can be seen in experiments 8 and 9 (table 2) in which some of the most extreme cardiac output variations from the mean of 42 animals were obtained. When these values (ca 1.99 and 3.50) were initially obtained suspicions of error in determination were aroused. However, when these values were constantly reproduced in repeated measurements, suspicions were allayed. It is easily recognized that repetitive control measurements are essential in laboratory animals, varying as they do in age, previous physical existence, nutritional state and blood pressure levels. It is in its ability to provide repetitive cardiac output values quickly for consideration by the investigator that the *modified Stewart method* excels other procedures.

(C) *The total peripheral resistance.* In accordance with the practices of the Munich school, the total peripheral resistance (TPR), calculated in accordance

with Poiseuille's law, can be computed in absolute units (A.U.) by the equation
$$\text{T.P.R.} = \frac{\text{mean pressure} \times 1332}{\text{cardiac output}/\text{sec.}} = \frac{\text{dynes/sec.}}{\text{cm.}^5}.$$
 Such units have been tersely designated as absolute units (A.U.) of total peripheral resistance in this laboratory. The only merit of such conversion rests in the easy comparison with values reported by others. The number of actual values and variations reported for intact anesthetized dogs is very meager. A summary of the results obtained under widely varying experimental conditions has been recently published from this laboratory (19). With the exception of a few determinations by Böger, who reported a range of 4020–4470 A.U. for a few intact dogs, all of these results were obtained on extensively operated dogs with open chests and in some cases in heart-lung-dog preparations.

Calculations made in 145 tests on 42 dogs of different sizes are included in the data of tables 2 and 3. The extreme ranges were from 3100 to 9400 A.U. However, 105 of the 145 determinations fell within the range of 3600 to 5400 A.U. with a surface area range of 0.6 to 1.10 sq. m. or a weight range of 12 to 31 kgm. In view of the small dimensions represented by the absolute units, the total resistance to outflow from all branches of the aorta is remarkably constant from dog to dog. While the possible effects of anesthesia on natural T.P.R. cannot be evaluated, the fact that 6 dogs anesthetized with chloralose and 3 with nembutal showed no essentially different trends makes it probable that 3600 to 5400 A.U. can be taken as approximate ranges for normal dogs with which alterations in experimental hypo- and hypertension may be compared in chronic dog experiments.

As summarized in table 5, the average T.P.R. for medium-sized dogs was 5570 A.U., that of large dogs, 4630 A.U. and of extra large dogs, 4230 A.U. Although there appears to be a slight correlation between T.P.R. and the size of the animal, the latter is not as constant as might be expected. Occasionally, smaller dogs revealed a smaller T.P.R. than larger ones, e.g., compare the smaller dogs 16, 18 and 35 with the larger dogs 3, 4, 7, 8, 21 and 24 in tables 2 and 3. Such findings make it questionable whether the practice of introducing surface area factors into computations of total peripheral resistance as suggested by Bazett et al. (20) serve to make them any more exact.

SUMMARY

1. Though perfectly sound in principle, the procedure for determining cardiac output as proposed by G. N. Stewart was found to invite so many technical inaccuracies that the values could not be regularly relied upon. Much effort was directed with the aim of overcoming these weaknesses.

2. The technical procedures were improved by (a) proper adjustment of the concentration and rate of infusion of the NaCl solution so that the salt appeared in sufficient concentration in the femoral blood stream to be easily detected without at the same time provoking any abnormality of cardiac action and a consequent artificial change in cardiac output, (b) technical and instrumental improvements for detecting the arrival of the uniform blood-NaCl mixture in a

femoral artery, (c) advancements in the technique of withdrawing a sample of the uniform mixture for electro-analysis and (d) improvement in the accuracy of electro-titration of the control sample to match the blood-NaCl sample.

3. As a result of the above expedients, the method has been improved to the extent that constantly reproducible measurements of cardiac output were obtained in 145 tests on 42 dogs. Furthermore, statistical analyses revealed that the standard deviation from the mean of these measurements was smaller than any which have been reported for dogs by other investigators. Comparisons of values obtained almost simultaneously by this *modified Stewart method* and by the more generally accepted Fick method were as comparable as could be expected in both normal and hypotensive dogs.

4. In 20 dogs, in which arterial blood pressure remained very stable, the mean *circulatory index*, expressed in liters/sq. m./min. was 2.77 and the average of the individual variations was only 0.05. In the remaining 22 dogs, in which arterial pressures fluctuated somewhat (18; S.D. ± 13 mm. Hg) the *circulatory index* was of the same order of magnitude (2.94), but the average of the individual variations was greater (0.19). There appears therefore to be a definite relation between the constancy of consecutive measurements to be expected and the stability of arterial blood pressure.

5. Although it appears from the results of this and other investigations that the control *circulatory index* of anesthetized mongrel laboratory dogs generally ranges from 2.5 to 3.3, such values cannot be considered as sufficiently restricted to justify their adoption as controls for any given experiment. On any occasion where it is anticipated that the proposed experimental procedure will modify the cardiac output, it is absolutely essential that several consistent control measurements be obtained. The *modified Stewart method* excels in its ability to provide quickly such repetitive cardiac output values for consideration by the investigator.

6. An analysis of these and previously reported data indicates that equivalence in values for the *circulatory index* in man and dog, or for that matter, among any of the animal species has not been adequately demonstrated.

7. The total peripheral resistance of the intact dog under control conditions, concerning which only very meager information has been published, ranged in these dogs from 3100 to 9400 A.U. In 105 of 145 measurements, however, it fell within narrower limits (3600-5400 A.U.). In certain chronic experiments in which control T.P.R. values cannot be obtained prior to the institution of experimental procedures (say states of hypotension or hypertension), the above range for control determinations offers some guide as to the extent to which T.P.R. changes may be concerned. Since little correlation was observed between the total peripheral resistance and body surface area, the advantage of introducing surface area into the equation for computing T.P.R. seems dubious.

It is a pleasure to express my gratitude to various colleagues for the services they have rendered in this investigation; to Dr. Frank Hovorka, professor of physical chemistry, for advice pertaining to conductivity measurements in general; to Dr. Harold Green of this department for his constructive criticisms

and suggestions pertaining to some of the technical problems, and finally; to Doctor Middleton and the following medical students (Messrs. Huizenga, Brofman, Antos and Dworkin) who at various times participated in the conduction of these experiments.

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A COMPARISON OF THE RENAL REABSORPTIVE PROCESSES FOR SEVERAL AMINO ACIDS

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A study of the renal mechanism for the reabsorption of glycine amino nitrogen in the dog (Pitts, 1943) has shown that there exists, at high plasma concentrations, a limiting maximal rate of tubular transfer similar to that described for glucose (Shannon and Fisher, 1938). But the mechanism for glycine is different in that the limiting rate of reabsorption is approached gradually and the amount excreted becomes appreciable before the reabsorptive system is saturated. As a consequence, no sharp renal threshold exists for glycine amino nitrogen such as that described for glucose.

The partition of urinary nitrogen following the oral administration of various amino acids suggests that all may not be reabsorbed with equal efficiency (Kriss, 1939). In fact, the studies of Doty (1941) indicate that certain amino acids, closely related structurally, may be reabsorbed at widely divergent rates. But these studies have not been directed toward an analysis of differences in the renal reabsorptive processes, nor do they permit a decision as to whether a single or several reabsorptive mechanisms are operative. The present investigation, in which the reabsorptive processes for representative amino acids have been studied in the dog, is concerned with these questions. It has been found that the amino acids glycine, alanine, glutamic acid and arginine are in all probability reabsorbed by a common mechanism and that the rather considerable variations in their rates of reabsorption result from differences in their rates of combination with some cellular component common to the reabsorptive system for all.

METHODS. The chemical methods used in this investigation have been described fully in our previous communication (Pitts, 1943). Observations have been made on two trained female dogs, one of which was used in our experiments on glycine. The general procedures of arterial blood sampling, intravenous infusion and urine collection were identical to those previously described. Because of the large amounts of amino acid required to elevate the plasma concentration to the desired levels, our choice of the ones to investigate has been determined both by economic factors and by solubilities. With these limitations as the prime consideration, we have chosen glycine and *D*-alanine as representative of monoamino-monocarboxylic acids; *L*-glutamic acid, as a monoamino-dicarboxylic acid; and *L*-arginine, as a diamino-monocarboxylic acid. Glutamic acid was infused as the neutral mono-sodium salt and arginine as the neutral mono-hydrochloride. For comparative purposes we have included likewise a mixture of amino acids derived from hydrolyzed casein,¹ also neutralized to pH 7.3.

¹ The pure amino acids were purchased from Merck & Co.; the casein hydrolysate was obtained through the courtesy of Frederick Stearns & Co.

All amino acid analyses are expressed in terms of milligrams of alpha amino carboxyl nitrogen per 100 cubic centimeters or per minute. Thus by dividing by 14, all values may be converted to millimols per 100 cubic centimeters or per minute.¹ It should be pointed out that the specificity of the gasometric ninhydrin carboxyl method for amino acid following preliminary treatment with urease is such that our results are uninfluenced by changes in plasma or urine urea, ammonia or peptide concentration.

RESULTS. *The characteristics of the reabsorptive processes for the several amino acids.* Simultaneous measurements of the creatinine clearance and plasma and urine amino nitrogen concentrations permit the calculation of the amount of amino nitrogen reabsorbed as the difference between the amount filtered and the amount excreted. The amount filtered equals the product of the creatinine clearance (glomerular filtration rate) and the plasma amino nitrogen concentration; the amount excreted equals the product of urine flow and urine concentration. The characteristics of the reabsorptive process for each amino acid have been assessed by measuring the quantity reabsorbed at each of a series of amounts filtered. In table 1 are given the significant data from three experiments on dog 4, comparing reabsorption of *D*-l alanine, *l*+ glutamic acid and *l*+ arginine. In each experiment 5 solutions containing constant amounts of creatinine and progressively increasing amounts of amino acid were infused at constant rate. Infusions were started 20 minutes before the beginning of each pair of experimental periods. Prior to the first infusion a control blood sample was taken. As a result of a standard diet and a constant preliminary period of fasting (16 hrs.) these control plasma amino nitrogen concentrations all fell within the limits of 3.5 and 4.0 mgm. per 100 cc. Under these conditions excretion is negligible, amounting to 0.02 to 0.05 mgm. per min. (Pitts, 1943). Thus at even the lowest rates of administration of the three amino acids, significant amounts were excreted (note the first pair of experimental periods in expts. 30, 31, and 33). As the amount filtered was increased by further raising the plasma concentration the amounts reabsorbed and excreted both increased. Increased reabsorption of alanine was considerable; of glutamic acid, moderate; and of arginine, slight. Experiments were designed to cover equivalent ranges of filtered amino nitrogen for each amino acid studied. The failure to attain this end resulted largely from reduction in glomerular filtration rate at high plasma concentrations. This reduction was most marked in experiments with glutamic acid, arginine and hydrolyzed casein. It should be emphasized that the depression of renal function is temporary and associated with other evidence of toxicity of a transient character (Pitts, 1943).

The results of all our experiments on dogs 4 and 1 are summarized in figures 1 and 2. In figures 1A and 2A, the quantity of the several amino acids reabsorbed is plotted as a function of the quantity filtered. In figures 1B and 2B, the quantity excreted is similarly plotted. Both qualitatively and quantitatively there is good agreement in the several families of curves derived from experiments on the two dogs. The amounts of the various amino acids reabsorbed stand in decreasing order of glycine, alanine, glutamic acid and arginine at all amounts

TABLE 1

Experiments on a normal dog which show the relationship between the amount of alpha amino nitrogen filtered and the amounts reabsorbed and excreted for the amino acids alanine, glutamic acid, and arginine

Dog 4, 16.8 kgm., S.A. 0.71 sq. m.

EXPER. NO.	GLOMER-ULAR FILTRATION RATE	URINE FLOW	AMINO NITROGEN					CLEARANCE RATIO: AMINO-N CREATININE
			Plasma conc.	Urine conc.	Filtered	Excreted	Reabsorbed	
	cc./min.	cc./min.	mgm. %	mgm. %	mgm./min.	mgm./min.	mgm./min.	
30		Control	3.63					
Infusion 1.5% alanine at 5 cc./min.								
	71.9	5.3	5.74	9.58	4.13	0.51	3.62	0.12
	69.0	3.1	6.80	24.0	4.70	0.74	3.96	0.16
Infusion 3.0% alanine at 5 cc./min.								
	74.4	3.3	10.4	58.6	7.73	1.94	5.79	0.25
	75.5	4.3	12.1	62.1	9.13	2.68	6.45	0.29
Infusion 5.0% alanine at 5 cc./min.								
	89.5	7.2	19.6	89.9	17.5	6.48	11.0	0.37
	92.7	8.5	22.9	103	21.2	8.75	12.5	0.41
Infusion 8.0% alanine at 5 cc./min.								
	96.8	9.9	35.9	180	34.8	17.8	17.0	0.51
	92.5	9.5	41.9	212	38.8	20.2	18.6	0.52
Infusion 12% alanine at 5 cc./min.								
	81.8	10.8	62.0	280	50.8	30.2	20.6	0.60
	84.3	13.0	69.3	279	58.5	36.3	22.2	0.62
31		Control	3.64					
Infusion 0.75% glutamic acid at 10 cc./min.								
	78.1	8.0	5.41	9.11	4.22	0.73	3.49	0.17
	71.6	6.0	6.09	19.0	4.36	1.14	3.22	0.26
Infusion 1.5% glutamic acid at 10 cc./min.								
	76.7	5.7	11.2	77.1	8.59	4.39	4.20	0.51
	80.6	8.1	14.3	72.7	11.5	5.89	5.61	0.51
Infusion 2.5% glutamic acid at 10 cc./min.								
	77.6	7.8	23.1	159	17.9	12.4	5.50	0.69
	72.2	7.1	25.3	160	18.3	11.4	6.90	0.62
Infusion 4.0% glutamic acid at 10 cc./min.								
	70.2	7.5	37.5	253	26.3	19.0	7.30	0.72
	63.7	7.66	42.5	363	27.1	20.2	6.90	0.74
Infusion 6.5% glutamic acid at 10 cc./min.								
	57.6	10.1	62.5	278	36.0	28.1	7.90	0.78
	51.7	10.2	70.4	289	36.4	29.5	6.90	0.80

TABLE 1—*Concluded*

EXPER. NO.	GLOMER- ULAR FILTRATION RATE	URINE FLOW	AMINO NITROGEN					CLEARANCE RATIO: AMINO-N CREATININE
			Plasma conc.	Urine conc.	Filtered	Excreted	Reabsorbed	
	cc./min.	cc./min.	mgm. %	mgm. %	mgm./min.	mgm./min.	mgm./min.	
33		Control	3.70					
Infusion 1% arginine at 10 cc./min.								
	82.1	11.4	5.17	4.81	4.25	0.55	3.70	0.13
	75.5	11.7	5.35	7.44	4.04	0.87	3.17	0.22
Infusion 2% arginine at 10 cc./min.								
	77.5	9.4	8.41	23.7	6.52	2.22	4.30	0.34
	73.6	9.0	9.06	25.3	6.68	2.28	4.40	0.34
Infusion 4% arginine at 10 cc./min.								
	71.5	11.7	17.4	74.3	12.5	8.70	3.80	0.70
	70.5	13.5	19.6	75.0	13.8	10.1	3.70	0.74
Infusion 6% arginine at 10 cc./min.								
	59.8	12.6	30.3	113	18.1	14.2	3.90	0.78
	58.2	13.7	33.9	113	19.7	15.5	4.20	0.78
Infusion 8% arginine at 10 cc./min.								
	51.6	16.6	47.3	122	24.5	20.2	4.30	0.83
	49.8	18.0	52.5	123	26.2	22.1	4.10	0.85

filtered. The amount of casein hydrolysate reabsorbed lies between glycine and alanine in the lower range of the curve and between alanine and glutamic acid in the higher range. The amounts of the several amino acids excreted obviously stand in reverse order.

Glycine and alanine, of the amino acids studied, are treated in most nearly the same fashion by the kidney. The alanine used was the racemic mixture and it is possible that the differences between the curves for glycine and alanine result in large part from a relative deficiency in reabsorption of the unnatural form of alanine (Bliss, 1941). Unfortunately, we have been unable to obtain either by purchase or preparation, sufficient quantities of resolved alanine to test the two forms separately. However, simple calculations from the data of table 1, experiment 30, show that some of the unnatural form of alanine must be reabsorbed. Reabsorption of alanine, like glycine, increases as the amount filtered is increased. Alanine would appear also to be reaching a limiting maximal rate of transfer, a limit which appears to be the same as that for glycine. Attempts to test the point directly by raising plasma alanine to very high levels were unsuccessful, for they were attended by serious reductions in glomerular filtration rate. The extrapolation of the alanine curve in figure 2A toward the glycine curve is based on the last two points obtained in another experiment on the same dog (see fig. 3A). For both dogs, however, the data would seem to justify extrapolation of the two curves toward the same limit.

The reabsorption of glutamic acid and arginine is much less complete than that of glycine and alanine, as is evident from figures 1 and 2. In fact, these figures somewhat exaggerate the reabsorption of all amino acids at low levels and especially that of glutamic acid and arginine. This results from the fact that figures for total plasma and urine amino nitrogens are used in making the calculations, and at low plasma concentrations a large fraction of the amino nitrogen

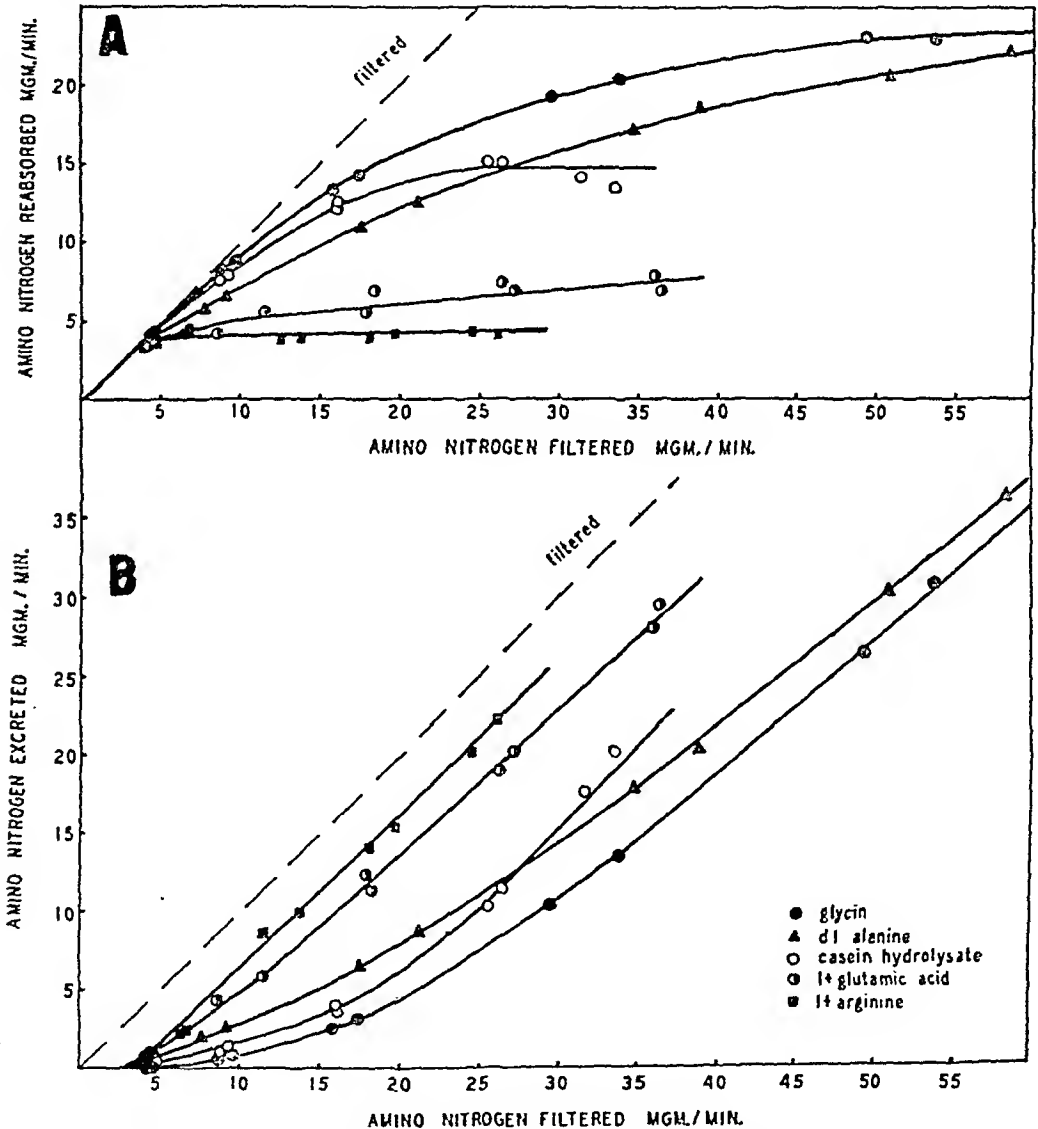


Fig. 1. A comparison in dog 4 of the renal reabsorptive and excretory processes for alpha amino nitrogen derived from glycine, alanine, glutamic acid, arginine and hydrolyzed casein.

reabsorbed is that which is normally present in the blood. Especially is this true in the glutamic acid and arginine experiments. However, the reabsorption of glutamic acid quite obviously continues to increase over the range studied and would seem to be approaching no readily apparent limit. While the reabsorption of total amino nitrogen in the arginine experiment would appear to have reached a plateau over a considerable portion of the range of observation, inspection of

table 1, experiment 33, suggests that in reality reabsorption of arginine itself continues to increase gradually. As is evident from experiment 33, or the lowest curve in figure 1A, an apparent reabsorptive limit of some 4 mgm. per min. of total amino nitrogen is attained in the last 8 experimental periods. In the earlier

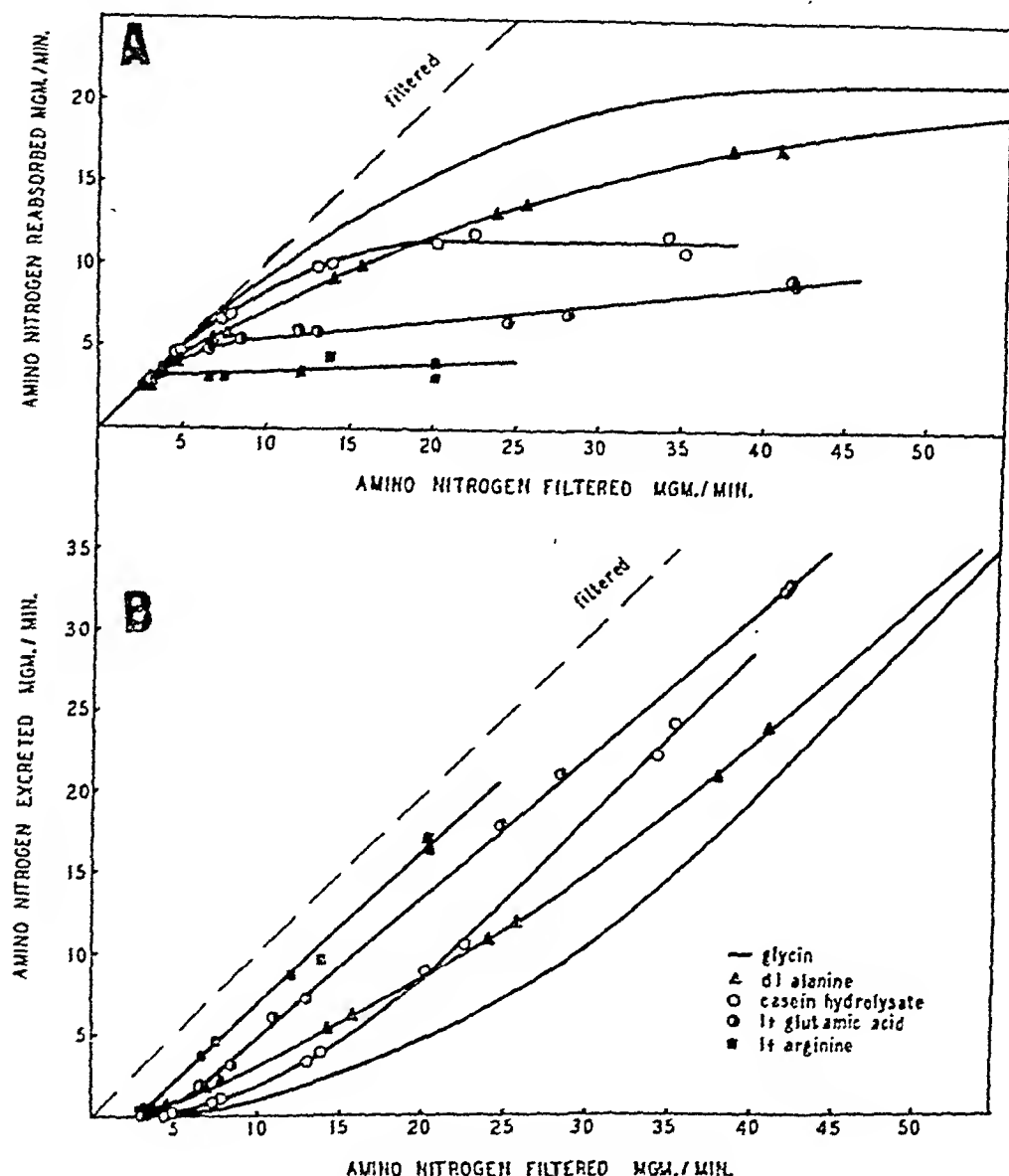


Fig. 2. A comparison in dog 1 of the renal reabsorptive and excretory processes for alpha amino nitrogen derived from glycine, alanine, glutamic acid, arginine and hydrolyzed casein. The curve for glycine is taken from the composite data of figure 1 (Pitts, 1943).

periods, most of the reabsorbed amino nitrogen is made up of that normally present in the plasma. In the later periods the decrease in filtration rate (evident in table 1, expt. 33) reduces the amount of this normal amino nitrogen available for reabsorption. Hence in the last periods the 4 mgm. per min. reabsorbed must consist largely of arginine nitrogen.

Whether the reabsorptive maximum attained in the experiments with hydrolyzed casein is more apparent than real cannot be stated definitely, although it is our impression that it does not represent a true cellular limitation of reabsorptive capacity. Both experiments on hydrolyzed casein (figs. 1 and 2) were complicated in the last periods by serious depression in glomerular filtration rate. If this resulted from closure of some glomeruli, it would reduce the number of tubules contributing to the reabsorptive process and hence impose an apparent limitation on the reabsorptive capacity of the system as a whole. To what extent this same factor may play a rôle in the experiments on glutamic acid and arginine cannot be stated.

Stability of the reabsorptive processes. The validity of comparisons of reabsorptive processes in such a series of experiments depends upon the stability of the systems under investigation. The general qualitative agreement of the results obtained in two dogs is an argument in favor of such stability. Direct evidence has been obtained by repetition of experiments with alanine and glutamic acid after intervals of 8 to 10 weeks. In figure 3A the amounts of alanine reabsorbed and excreted are plotted against the amount filtered. The hollow symbols are the results obtained in the first experiment; the solid symbols, those obtained 8 weeks later. In figure 3B are plotted two similar experiments with glutamic acid separated by intervals of 10 weeks. The reproducibility of the results obtained is indicative both of stability of the reabsorptive processes and of the lack of any chronic damage to the kidneys resulting from repeated administration of large amounts of amino acid.

Evidence that the several amino acids are reabsorbed by a common mechanism. That the characteristics of the reabsorptive processes are different for the several amino acids studied is evident from figures 1 and 2. Such differences as are observed might be reflections of the way these amino acids are handled by a common mechanism, or might indicate that several discrete mechanisms are involved. The fact that creatine and glycine are reabsorbed by a common mechanism and that saturation of the reabsorptive system with glycine reversibly blocks the reabsorption of creatine² (Pitts, 1943) has been used to distinguish between these two possibilities. In the experiments on dog 1 presented in table 2, creatine was administered in such amounts that appreciable quantities were reabsorbed. The administration of alanine in increasing amounts (expt. 35) progressively reduced, from its control value of 4.7 mgm. per minute, the quantity of creatine reabsorbed. At plasma amino nitrogen concentrations approaching those necessary for saturation of the reabsorptive mechanism, creatine reabsorption was reduced to zero. Since both alanine and glycine reversibly block creatine reabsorption, we infer that the two amino acids are reabsorbed by the same mechanism.³

² We have been forced to use this indirect method because of the lack of specific chemical methods capable of distinguishing glycine, alanine and glutamic acid.

³ Competition of amino acids and creatine for a common renal reabsorptive mechanism may well be a determining factor in the creatinuria of hyperthyroidism, in which condition plasma amino acids are elevated.

A similar experiment (expt. 38) was performed with creatine and glutamic acid. The somewhat higher plasma creatine concentrations account for greater reab-

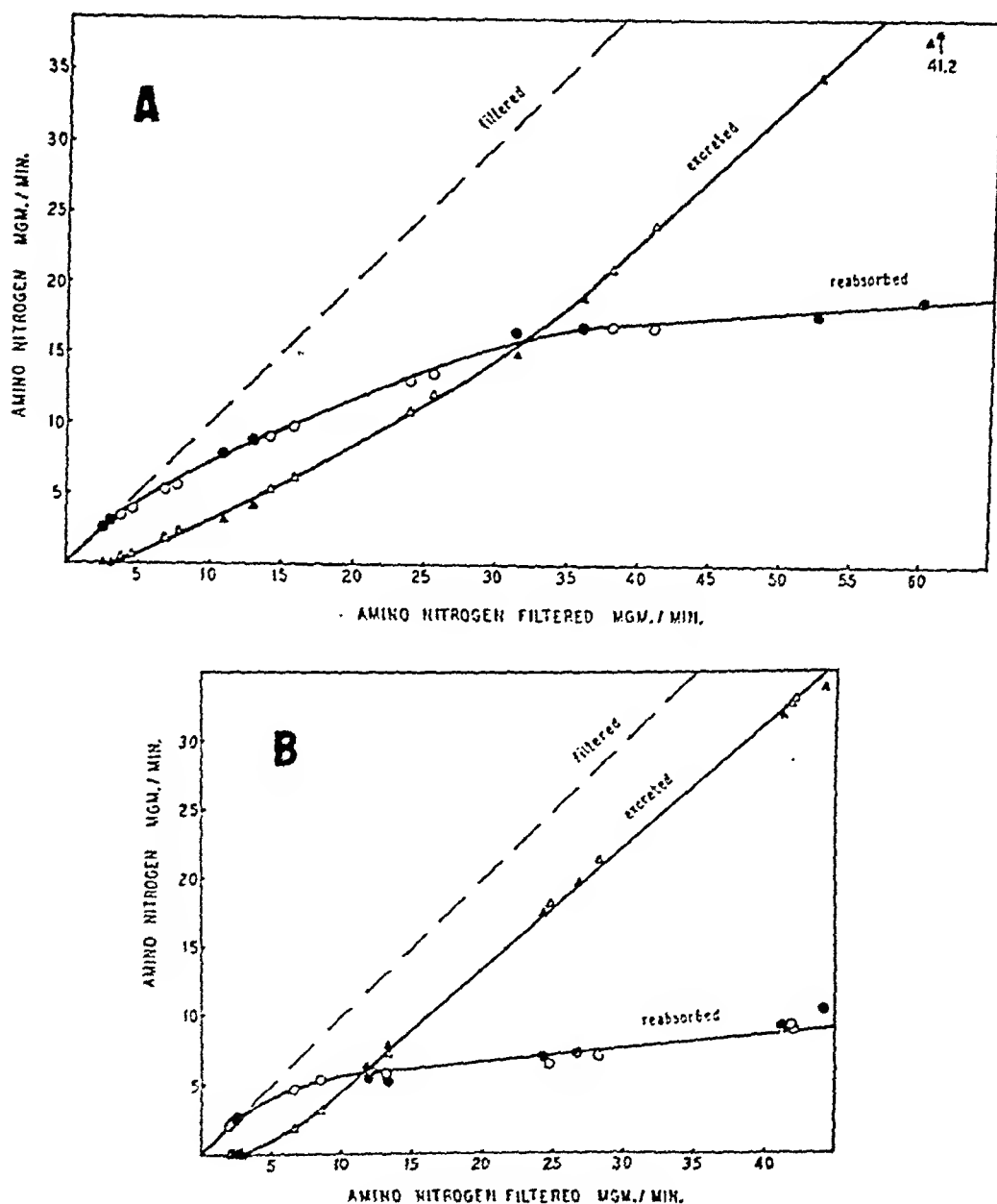


Fig. 3. Experiments on dog 1 illustrating the stability of the renal reabsorptive processes for alanine and glutamic acid nitrogen. *A*, reabsorption and excretion of alanine nitrogen as a function of the quantity filtered in 2 experiments separated by an interval of 8 weeks. Hollow symbols, first experiment; solid symbols, repetition after 8 weeks. *B*, reabsorption and excretion of glutamic acid nitrogen as a function of the quantity filtered in 2 experiments separated by an interval of 10 weeks. Hollow symbols, first experiment; solid symbols, repetition after 10 weeks.

sorption in the control periods of this experiment than in the previous one. The administration of glutamic acid definitely depresses, though it does not completely

TABLE 2

Experiments on a normal dog which illustrate competition between creatine and alanine and glutamic acid nitrogen for a common reabsorptive mechanism

Dog 1, 18.7 kgm., S.A. 0.72 sq. m.

EXPER. NO.	GLOMER- ULAR FILTRATION RATE	AMINO NITROGEN			CREATINE		
		Plasma conc.	Excreted	Reabsorbed	Plasma conc.	Excreted	Reabsorbed
Infusion 1.5% creatine; 0% alanine at 5 cc./min.							
35	cc./min.	mgm. %	mgm./min.	mgm./min.	mgm. %	mgm./min.	mgm./min.
	85.0	3.63	0.02	3.06	51.5	38.9	4.9
	80.5	3.18	0.03	2.53	54.1	39.1	4.5
							—
							4.7
Infusion 1.5% creatine; 4% alanine at 5 cc./mi .							
	91.2	12.0	3.10	7.80	55.0	47.6	2.6
	88.5	14.7	4.20	8.80	56.8	47.2	3.1
							—
							2.9
Infusion 1.5% creatine; 8% alanine at 5 cc./min.							
	92.0	34.1	15.0	16.4	63.8	57.6	1.1
	89.6	40.2	19.1	17.0	67.3	59.2	1.2
							—
							1.2
Infusion 1.5% creatine; 12% alanine at 5 cc./min.							
	83.1	63.2	34.8	17.8	68.5	56.9	0.0
	80.5	74.5	41.2	18.8	70.7	57.0	0.0
							—
							0.0
Infusion 1% creatine; 0% glutamic acid at 10 cc./min.							
38	80.8	3.37	0.02	2.70	70.8	49.4	7.9
	87.0	3.03	0.03	2.60	70.1	53.5	7.5
							—
							7.7
Infusion 1% creatine; 2.5% glutamic acid at 10 cc./min.							
	87.9	13.4	6.20	5.60	75.7	59.6	6.9
	82.7	16.1	8.00	5.30	80.6	62.0	4.8
							—
							5.9
Infusion 1% creatine; 5.0% glutamic acid at 10 cc./min.							
	77.6	31.1	17.3	6.90	85.0	61.3	4.7
	73.9	36.1	19.6	7.10	90.0	61.5	5.0
							—
							4.9
Infusion 1% creatine; 7.5% glutamic acid at 10 cc./min.							
	74.0	55.5	31.8	9.30	94.9	65.8	4.5
	69.7	63.4	33.6	10.60	99.3	65.0	4.4
							—
							4.5

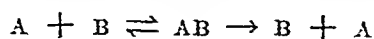
block creatine reabsorption. The greatest amount of glutamic acid nitrogen reabsorbed in this experiment is roughly 40 per cent of the maximal reabsorptive capacity for glycine or alanine nitrogen. The reabsorption of creatine is depressed roughly by an equivalent 40 per cent. This experiment lends credence to the view that glycine, alanine, and glutamic acid are reabsorbed by a single mechanism. It also indicates that all might well approach the same limiting maximal rate of reabsorption if the amount filtered could be sufficiently increased. This latter statement derives from the fact that reabsorption of glutamic acid nitrogen in amounts equal to 40 per cent of the limiting reabsorptive capacity for glycine or alanine blocks creatine reabsorption by 40 per cent. It is inferred that complete blockage of creatine reabsorption could be effected only by the reabsorption of glutamic acid in amounts equivalent to the limiting capacities for glycine and alanine. Presumably this would require an amount filtered some two to three times that which it has been possible to attain experimentally.

Experiments of a similar nature with creatine and arginine were negative, the effect of arginine on creatine reabsorption, if any, being so slight as to be within the limits of experimental error. Providing that arginine is reabsorbed by the same mechanism as glycine, alanine and glutamic acid, this becomes a significant finding. The amount of arginine reabsorbed at the highest amount filtered is relatively insignificant in comparison with the maximal tubular reabsorptive capacity for glycine. As a consequence it would be predicted that arginine would have little effect on creatine reabsorption within the range studied. To determine whether arginine is reabsorbed by the same mechanism as the other amino acids studied, we have maintained a constant plasma arginine⁴ concentration and have tested the effect upon arginine reabsorption of increasing the plasma glycine. The results of this experiment are given in table 3. A previous experiment in which glycine was given at higher rates of infusion was complicated by a serious glomerular shut-down. In the experiment of table 3 moderate elevation of plasma amino nitrogen by the infusion of glycine significantly depressed the reabsorption of arginine although it did not block it. While not conclusive, the evidence suggests that arginine may be reabsorbed by that same renal mechanism which reabsorbs glycine, alanine, glutamic acid and creatine. If so, the negative effects of arginine on creatine reabsorption within the range studied would indicate that only an insignificant fraction of the total reabsorptive capacity is occupied by arginine to the exclusion of creatine. It is within the realm for speculation, then, to make the experimentally unjustified extrapolation of the arginine curve toward a common reabsorptive limit with the other three amino acids.

DISCUSSION. Shannon and Fisher (1938) have developed the thesis that active renal reabsorptive processes depend upon the combination of the reabsorbed

⁴ Arginine analyses were performed by the colorimetric method of Dubnoff (1941) and are expressed in terms of milligrams of alpha amino arginine nitrogen. The amino nitrogen analyses of course include normal plasma amino nitrogen, arginine nitrogen, and glycine nitrogen.

material, A, in the tubular lumen with some stable component, B, present in the tubular cells in fixed amount, according to the scheme:



The decomposition of the complex AB delivers the reabsorbed material into the peritubular interstitial fluid. This latter reaction has the characteristics of a first

TABLE 3

Experiment on a normal dog which illustrates competition between arginine and glycine nitrogen for a common reabsorptive mechanism

Dog 4, 16.8 kgm., S.A. 0.71 sq. m.

EXPER. NO.	GLOMER- ULAR FILTRATION RATE	α -ARGININE NITROGEN			AMINO NITROGEN		
		Plasma conc.	Excreted	Reabsorbed	Plasma conc.	Excreted	Reabsorbed
Infusion 1% arginine; 0% glycine							
40	cc./min.	mgm. %	mgm./min.	mgm./min.	mgm. %	mgm./min.	mgm./min.
	77.0	1.68	0.51	0.79	5.30	0.67	3.41
	73.0	1.65	0.43	0.81	5.18	0.56	3.22
				0.80			3.32
Infusion 1% arginine; 2% glycine							
	83.2	1.29	0.34	0.73	10.7	1.41	7.49
	86.9	1.29	0.42	0.70	11.0	1.98	7.57
				0.72			7.53
Infusion 1% arginine; 4% glycine							
	96.6	1.14	0.49	0.61	16.8	5.0	10.8
	99.8	1.21	0.59	0.62	18.1	6.6	11.5
				0.62			11.2
Infusion 1% arginine; 6% glycine							
	105	1.14	0.62	0.58	24.3	11.8	13.7
	98.2	1.22	0.67	0.53	29.3	13.7	15.1
				0.56			14.4

order process, for if sufficient A is present to transform all of B into AB, the rate of reabsorption of A reaches a constant limiting value independent of the concentration of A and determined solely by the velocity of decomposition of AB, assuming of course that total B ($B + AB$) is constant. Such a concept accounts adequately for the limited reabsorptive capacity of the kidney for both glucose and glycine. However, as the plasma concentration of glycine is increased this limiting reabsorptive maximum is attained rather gradually, a fact explained by assuming that

the velocity of decomposition of AB is high in relation to the rate of attainment of equilibrium in the reaction by which it is formed (Pitts, 1943). Thus when A (glycin) is present in amounts insufficient to saturate the reabsorptive mechanism, its rate of reabsorption will be determined both by its concentration and by its specific velocity of combination with substance B.

The rather considerable differences in the reabsorptive processes for the amino acids which we have studied might conceivably result from 1, independence of distinct reabsorptive mechanisms for the several amino acids; or 2, differences in the way the several amino acids are handled by a common reabsorptive mechanism. In the latter circumstance both velocity of formation of the B complex and velocity of its decomposition might enter into the determination of the characteristics of the reabsorptive process.

The evidence that a single reabsorptive mechanism is concerned with the tubular transport of glycin, alanine, glutamic acid and arginine is fairly conclusive. It rests basically on the demonstration that saturation of the renal reabsorptive mechanism with glycin or alanine blocks the simultaneous reabsorption of creatine. Since both amino acids compete with creatine during reabsorption, they must of necessity be reabsorbed by a single mechanism. Reabsorption of glutamic acid in less than saturation amounts reduces creatine reabsorption in direct proportion to the extent to which it occupies the common mechanism. The reabsorption of arginine is similarly reduced if glycin competes simultaneously for the reabsorptive mechanism. A single cellular element, B, is therefore common to the reabsorptive systems for the four amino acids and creatine as well. One must look to differences in the kinetics of formation or decomposition of the various B complexes for an explanation of the dissimilar characteristics of the reabsorptive processes.

If the velocities of decomposition of the several B complexes were identical, then the maximal reabsorptive capacities would be the same for a given animal for all amino acids, although the courses by which that maximum is attained might differ as a function of the effective rates of complex formation. While by no means conclusive, the data suggest this explanation as a *distinct possibility*. Thus from figures 1A and 2A it is evident that glycin and alanine approach essentially the same maximal rate of reabsorption at high plasma concentrations. From table 2 it is evident that glutamic acid, at such a plasma concentration that it is reabsorbed at a rate 40 per cent of that maximal for glycin, blocks creatine by an equivalent 40 per cent. It is inferred that if plasma glutamic acid could be elevated sufficiently to block creatine reabsorption completely, glutamic acid also would reach that same maximal rate of reabsorption attained by glycin and alanine. The same line of argument may be applied to the reabsorption of arginine, although here the evidence is almost entirely inferential.

If this is a correct interpretation of the data, it follows that the differences between the reabsorptive processes for the four amino acids are largely dependent on differences in the effective rates of formation of their B complexes. The lower the effective rate of formation of the complex the more gradually is the reabsorptive maximum attained. The rate is greatest for glycin and least for arginine

and creatine. It is interesting that the rates are inversely related to molecular weight, but it is doubtful if this is of significance for Bliss (1941) has shown that differences exist between the reabsorptive processes for *d* and *l* alanine.

SUMMARY

1. A comparison has been made in the dog of tubular reabsorption of amino nitrogen at various arterial plasma levels of glycine, alanine, glutamic acid, arginine and the amino acids of hydrolyzed casein.

2. The amounts of the various amino acids reabsorbed stand in decreasing order of glycine, alanine, glutamic acid and arginine at all amounts filtered. The amount of casein hydrolysate reabsorbed lies between glycine and alanine in the lower range of plasma concentration and between alanine and glutamic acid in the upper range.

3. Glycine, alanine, glutamic acid and arginine are reabsorbed by a common renal mechanism.

4. The rather considerable differences in their rates of reabsorption probably result from differences in their rates of combination with some cellular component common to the reabsorptive system for all.

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THE RELATION BETWEEN URIC ACID EXCRETION AND HIPPURIC ACID SYNTHESIS IN MAN

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In view of the significance attributed to the synthesis of hippuric acid from ingested sodium benzoate as a test of liver function in man (1, 2, 3), it was considered desirable to elucidate the uric acid retention which occurs during hippuric acid synthesis. That uric acid retention does occur was observed by Lewis and Karr (4), and later confirmed by Swanson (5) and Quick (6) whose report also includes a review of the literature pertaining to the synthesis and excretion of hippuric acid in man.

A decrease in uric acid excretion has also been observed following ingestion of lactic acid. Contrariwise, an increase follows ingestion of pyruvic acid—an oxidation product of lactic acid (7). In the light of these observations, determinations of lactic acid and pyruvic acid in the blood were included in the present study.

METHODS. The subjects of the study herein reported were male schizophrenic patients with no evidence of physical diseases. Two subjects who developed a destructive liver disease with jaundice during arsenical therapy for neurosyphilis are also included in this report since, in their cases, the extremely low rate of hippuric acid synthesis was advantageous in demonstrating the influence of sodium benzoate on uric acid retention. All subjects were studied in a fasting state and were reclining in bed during the period of the observation.

The experimental conditions were arranged on a pattern similar to that of the liver function test devised by Quick (1). The subjects voided at approximately 7 a.m. A urine sample was collected at approximately 8 a.m. and immediately (within one minute) followed by the ingestion of 80 mgm. of sodium benzoate per kilogram body weight diluted in approximately 30 ml. of water. In two subjects the amount of sodium benzoate ingested was 106 and 64 mgm. per kgm. body weight respectively. One drop of oil of peppermint was added to each dose as a corrigent of taste. Four urine samples were collected at hourly intervals following the ingestion of benzoate. Blood samples from a cubital vein were obtained with a minimum amount of stasis preceding the ingestion of sodium benzoate, and 1½ and 3½ hours following the ingestion. One glass of water was ingested 5 to 10 minutes previous to each voiding to facilitate urine formation. Only in one instance was the urine volume below 1 ml. per minute—the augmentation limit for uric acid excretion given by Brochner-Mortensen (8). In this instance, clearances based on the standard or maximum clearance formulae, as indicated by the urine volume, were used instead of excretion rates in the calculations described below.

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A portion of the blood samples was stabilized with iodoacetate and oxalate, precipitated within three minutes with trichloroacetic acid, and the filtrate analyzed for pyruvic acid by the method described by Bueding and Wortis (9) and for lactic acid by the Miller-Muntz technic as described by Barker and Summerson (10). One half-volume of each urine sample was used for the gravimetric determination of hippuric acid by the method of Quick (1) with the sodium chloride saturation recommended by Weichselbaum and Probst (11). Uric acid in urine and blood serum was determined with the photoelectric colorimeter according to the method of Folin (12).

The rate of synthesis of hippuric acid was measured from the amount of hippuric acid excreted during the hourly intervals. The value obtained for hippuric acid was converted into milligrams of sodium benzoate per kilogram body weight theoretically required for the construction of the amount of hippuric

TABLE 1
*Two representative studies of hippuric acid synthesis
with calculated factors as described in text*

SUBJECT	URINE SAMPLE	TIME	VOLUME	HIPPURIC ACID	SOD. BENZ. NOT SYNTHESIZED AT END OF PERIOD	URIC ACID	
						Mgm./ hr.	$\frac{U_0}{U_x}$
			ml.	gm.	mgm./kgm.		
F. M. Weight 55 kgm.	U ₀	6.45- 8.20	156			19.7	1.0
Sodium benzoate	U ₁	9.33	220	0.97	66	9.6	2.1
4.4 grams at 8:21	U ₂	10.22	76	0.78	55	5.0	3.9
a.m.	U ₃	11.19	276	1.00	40	5.0	3.9
	U ₄	12.20	87	0.88	27	8.9	2.2
E. P. Weight 64 kgm.	U ₀	6.20- 8.51	154			30.6	1.0
Sodium benzoate	U ₁	9.53	192	1.58	60	24.9	1.2
5.1 grams at 8:52	U ₂	10.54	126	1.52	41	9.4	3.3
a.m.	U ₃	11.52	242	1.91	17	24.0	1.3
	U ₄	12.52	168	0.79	7	25.6	1.2

acid excreted. Thus, in subject E. P. (table 1) weighing 64 kgm., the amount of hippuric acid excreted during the first hour—1.58 gram—represents 20 mgm. per kgm. body weight of the sodium benzoate ingested.

It was found expedient for the interpretation of uric acid retention to express the rate of synthesis in negative terms, i.e., in terms of sodium benzoate not synthesized up to a certain time. These values were obtained by subtracting the sum of values in milligrams of sodium benzoate per kilogram body weight synthesized and excreted up to the considered time from the value of sodium benzoate per kilogram body weight ingested at the beginning of the experiment. In subject E. P. (table 1) 60 mgm. (= 80 mgm. minus 20 mgm.) of sodium benzoate per kgm. body weight were still unaccounted for at the beginning of the second hour, 41 mgm. at the beginning of the third hour, 17 mgm. at the beginning of the fourth hour and 7 mgm. were not recovered as hippuric acid after

four hours of synthesis. Interpolations for the half-times of the hour used for correlations with uric acid retention were obtained by adding one-half of the sodium benzoate synthesized and excreted during the particular hour to the sodium benzoate synthesized during the foregoing periods.

RESULTS. The uric acid excretion decreased in all subjects after ingestion of sodium benzoate. The values given in table 1 are representative in this respect. In both subjects (cf. table) the uric acid excretion rate decreased gradually to a low level and subsequently returned toward the initial excretion rate, thus forming a peaked curve of retention. In subject E. P. the peak of uric acid retention occurred during the second hour following benzoate ingestion, while in subject F. M. the peak was at the end of the second or the beginning of the third hour period.

The peak of uric acid retention was similarly estimated in all subjects and it was found to occur at the end of the second hour period in most instances. With the exception of one case with severe liver disease (subject L. G., table 2), the peak was within the approximate range of one hour to $2\frac{1}{2}$ hours following the ingestion of benzoate.

In the third and fourth hour periods, the uric acid excretion rates were still at low levels in those subjects who synthesized hippuric acid slowly (subject F. M. in table 1), whereas a return to values bordering on the initial pre-experimental level was observed in subjects who synthesized hippuric acid rapidly (subject E. P. in table 1). It was found that the degree of uric acid retention was roughly proportional to the amount of ingested sodium benzoate which remained in the organism after deduction of the portion which had been excreted as hippuric acid up to the considered time. Only the values which followed the peak of uric acid retention showed this proportionality. Therefore, only values derived from the third and fourth hour periods were employed in the calculation of the correlation between the uric acid retention and the non-synthesized sodium benzoate which is graphically presented in figure 1. The uric acid retention is expressed in terms of the ratio of the uric acid excretion rate in milligrams per hour of the pre-experimental period to the excretion rate of the third or fourth hour period following ingestion of benzoate $\left(\frac{U_0}{U_3}, \frac{U_0}{U_4}\right)$. The retained benzoate is expressed in milligrams of sodium benzoate per kilogram body weight ingested and not excreted, synthesized to hippuric acid at the half-time of the respective periods. The correlation coefficient of these values is $+0.79 \pm 0.044$.

The serum uric acid level increased during the synthesis in 24 of 25 subjects. The increase, measured at the half-time of the fourth hour period and compared with the pre-experimental levels, ranged from 0.1 to 0.9 with an average of 0.5 mgm. uric acid per 100 ml. serum (subjects with liver disease not included).

The concentration of uric acid in the urine decreased to values lower than those of the serum in 17 of 25 of our subjects (cf. table 2). This observation seemed of interest in view of the unsolved problem in physiology of the mechanism of excretion of uric acid by the kidney.

TABLE 2
Hippuric acid syntheses by 10 subjects

SUBJECT	AGE	WEIGHT	URINE SAMPLES				BLOOD SAMPLES			
			Time of collection	Volume	Hippuric acid	Uric acid	Time of collection	Serum uric acid	Lactic acid	Pyruvic acid
		<i>kgm.</i>		<i>ml.</i>	<i>gm.</i>	<i>mgm./hr.</i>		<i>mgm./100 ml.</i>	<i>mgm./100 ml.</i>	<i>mgm./100 ml.</i>
T. G.	48	68	6.32- 8.31*	600		23.6	8.30	3.1	6.9	0.98
			9.32	295	0.67	13.1				
			10.32	132	0.72	6.6	10.07	3.1	9.2	1.00
			11.25	246	1.61	7.9				
			12.35	130	1.72	19.6	12.26	3.4	5.6	1.05
A. D.	38	54	6.24- 8.11*	410		24.8	7.44	4.4	5.0	0.80
			9.12	310	0.82	11.1				
			10.12	136	0.80	5.6	9.41	4.6	6.1	0.85
			11.12	240	1.24	7.5				
			12.14	240	1.17	28.9	11.44	4.8	5.9	0.96
E. S.	29	58	6.44- 8.01*	423		42.2	7.36	3.7	5.5	0.76
			9.02	386	1.00	14.5				
			10.03	270	1.29	9.8	9.36	3.7	7.3	0.62
			11.02	250	1.42	15.5				
			12.01	164	0.87	24.8	11.33	4.3	5.2	0.75
A. T.	47	61	6.45- 8.05*	378		27.2	7.49	4.5	5.3	0.61
			9.06	370	0.91	12.8				
			10.06	508	1.30	9.0	9.50	4.5	11.7	0.85
			11.06	352	1.39	11.8				
			12.04	126	0.60	15.7	11.43	5.1	6.4	0.65
R. W.	26	59	6.00- 8.34*	256		31.3	8.29	4.5	7.0	0.79
			9.33	252	1.03	13.6				
			10.33	176	1.35	7.0	10.07	5.0	6.6	0.63
			11.33	520	1.48	9.9				
			12.34	320	1.36	17.9	12.06	5.2	5.6	0.70
J. C.	23	49	6.20- 8.42*	291		22.6	7.55	4.9	7.3	0.84
			9.41	127	0.96	14.6				
			10.42	200	1.22	8.3	10.32	5.4	9.5	1.00
			11.42	298	1.42	13.1				
			12.42	167	0.91	17.2	12.21	5.8	9.0	0.72
J. R.	15	52	7.15- 8.09*	320		30.2	7.44	4.9	8.9	1.30
			9.10	248	1.00	11.9				
			10.13	239	1.68	10.0	9.45	5.1	8.3	1.16
			11.10	250	1.52	14.2				
			12.09	248	0.72	26.7	11.44	5.5	7.5	1.15
F. M.	44	66	6.55- 7.59*	458		29.2	7.34	4.7	6.6	1.09
			9.00	355	0.64	14.4				
			10.03	240	1.07	10.8	9.39	4.7	7.3	1.16
			11.00	246	1.33	10.6				
			12.00	216	0.65	17.7	11.38	5.4	5.4	0.96

TABLE 2—*Concluded*

SUBJECT	AGE	WEIGHT	URINE SAMPLES				BLOOD SAMPLES			
			Time of collection	Volume	Hippuric acid	Uric acid	Time of collection	Serum uric acid	Lactic acid	Pyruvic acid
		<i>kgs.</i>		<i>ml.</i>	<i>gm.</i>	<i>mgm./cc.</i>		<i>mgm./100 ml.</i>	<i>mgm./100 ml.</i>	<i>mgm./100 ml.</i>
K. F.	60	61	7.08- 8.18*	57		45.4	7.45	3.1	9.0	0.87
			9.10	72	0.24	23.8				
			10.10	80	0.43	9.1	9.48	3.4	11.0	0.91
			11.20	92	0.66	10.7				
			12.24	74	0.69	16.2	11.56	3.8	11.0	0.84
Same subject, following day			7.16- 8.00	32		42.3	7.36	3.3	10.5	0.84
L. G.	44	75	6.30- 8.53*	420		72.0	8.50	2.4	13.1	1.36
			9.54	420	No precipitate	41.3				
			11.26	500		16.3	10.54	3.8	13.1	1.74
			12.55	240		10.6	1.01	4.5	15.4	2.10
			Same subject 15 mos. later			85	7.50- 8.30*	158		33.7
			9.20	354	0.74	25.1				
			10.30	280	0.82	6.6				
			11.30	192	1.77	11.5				
			12.30	62	1.00	10.0				

All subjects ingested 80 mgm. of sodium benzoate per kgm. body weight except J. R. (102 mgm./kgm.) and F. M. (64 mgm./kgm.). The sodium benzoate was ingested at the time marked by an asterisk. Subjects K. F. and L. G. (first test) had toxic jaundice. Subject L. G. was recovered from jaundice at the time of the second test 15 months later.

In 21 of 24 subjects a moderate increase in blood lactic acid was observed. The average increase for the total group was 1.5 mgm. per cent at $1\frac{1}{2}$ hours after the ingestion of the benzoate. The standard error of the mean was ± 0.45 . The increase is considered significant as it would have been observed by chance less than once in one hundred occasions ($t = 4.61$, $P < 0.01$). In the samples collected $3\frac{1}{2}$ hours following ingestion of benzoate, the blood lactic acid levels were decreasing when compared to the $1\frac{1}{2}$ -hour values. This pattern of increase and decrease was singularly confined to the blood lactic acid, as it did not occur in the pyruvic acid and uric acid levels and in several other constituents (also cell volume) determined sporadically in individual cases.

The degree of increase in blood lactic acid had no correlation with the amount of hippuric acid excreted. However, the trend of the lactic acid shift paralleled the uric acid retention in the individual subjects (cf. table 2) and in the total group. The parallelism is apparent from table 3 in which the matched statistical values of sixteen subjects are presented. There was no correlation of the magnitude of the lactic acid shift with the degree of uric acid retention in the individual subjects or between the subjects, nor was the correlation of the absolute blood lactic acid levels with the uric acid excretion significant.

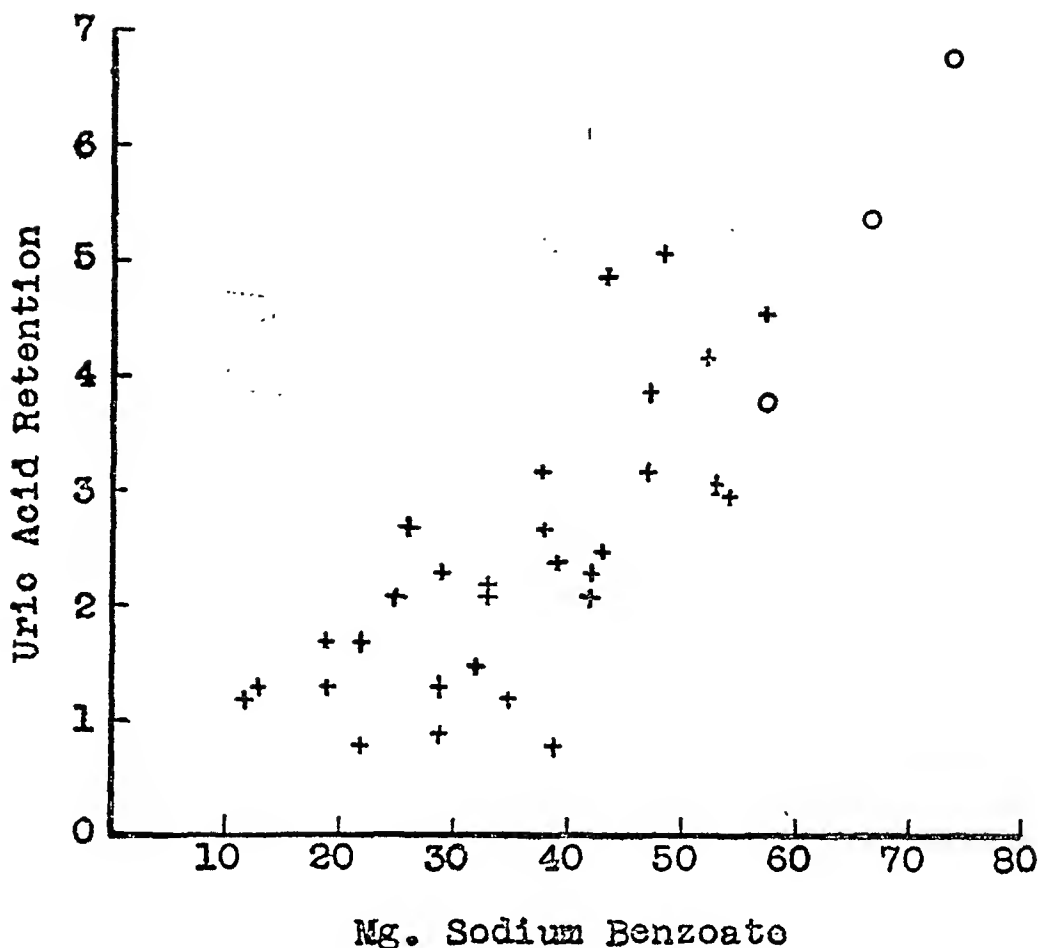


Fig. 1. Correlation between uric acid retention and retained sodium benzoate. Ordinates: Ratio of pre-test uric acid excretion rate over excretion rates for third or fourth hours following ingestion of sodium benzoate. $\left(\frac{U_0}{U_3}, \frac{U_0}{U_4}\right)$. Abseissae: Amount of sodium benzoate in mgm. per kgm. body weight not excreted as hippuric acid at the time corresponding to the indicated retention of uric acid. Correlation coefficient = $+0.79 \pm 0.044$. All subjects ingested 80 mgm. of sodium benzoate per kgm. of body weight. Circles represent subjects with toxic jaundice.

TABLE 3

Changes in blood lactic acid and pyruvic acid levels and in uric acid excretion during hippuric acid synthesis

Values represent averages of 16 subjects. Only subjects without evidence of physical disease represented

	LACTIC ACID		PYRUVIC ACID		URIC ACID EXCRETION	
	Mgm./100	Standard error of the mean	Mgm./100	Standard error of the mean	Mgm./hr.	Standard error of the mean
Before ingestion of sodium benzoate.....	6.2	± 0.29	0.83	± 0.038	29.0	± 2.39
1½ hours after ingestion	8.0	± 0.36	0.81	± 0.033	7.7	± 0.49
3½ hours after ingestion	6.6	± 0.32	0.81	± 0.038	20.2	± 2.14

COMMENT. The pattern of uric acid retention as it presents itself in our observations seems to depend on two factors. One is the resorption of the ingested benzoate from the gastro-intestinal tract, the other - the rate of inactivation of the benzoate by synthesis to hippuric acid. The sodium benzoate is absorbed from the intestinal tract faster than it is eliminated by synthesis to hippuric acid, and the excess of sodium benzoate in the organism effects retention of uric acid. When the amount of benzoate absorbed per unit of time equals that of benzoate removed by synthesis at the same time the retention of uric acid reaches its peak. Following the peak of retention the uric acid excretion rapidly increases. It seems probable that very little additional sodium benzoate is absorbed in this phase of the synthesis and that the uric acid excretion increases in proportion to the removal of the absorbed sodium benzoate by synthesis to hippuric acid. This post-absorptive period corresponds to the correlation graph presented in figure 1.

In conformity with this interpretation is the occurrence of some proportionality between the intensity of uric acid retention and the rate of hippuric acid synthesis during the initial period of the synthesis. An example of this proportionality is presented in table 1. The subject F. M. with a slow rate of synthesis has higher values for uric acid retention in the first two periods $\left(\frac{U_0}{U_1} 2.1, \frac{U_0}{U_2} 3.9\right)$ than the rapidly synthesizing E. P. (1.2 and 3.6 respectively). Apparently the subject with the slow rate of synthesis accumulates more benzoate than the subject who eliminates the benzoate by rapid synthesis—provided the absorption proceeds at an approximately equal rate. As a consequence, the retention of uric acid is higher in the subject with a slow rate of synthesis.

The final chemical phase of the synthesis, i.e., the phase that leads to the formation of hippuric acid, does not seem to be essential for the occurrence of uric acid retention. This contention is derived from the experiments of the subjects with destructive liver disease. A remarkable retention of uric acid occurred in these subjects even though very little hippuric acid was formed (cf. subjects K. F. and L. G. in table 2).

The process of excretion per se of hippuric acid by the kidney does not seem to have any relation to the uric acid retention. The retention of uric acid increased, reached its peak, and decreased even when the excretion of hippuric acid remained at a comparatively even rate throughout the synthesis. Additional evidence to this point was obtained when it was found that sodium hippurate, ingested and recovered in the urine in amounts comparable to those excreted during hippuric acid synthesis, did not cause a retention of uric acid similar to that found after the ingestion of sodium benzoate (unpublished observation).

The possibility that the observed changes in the blood lactic acid are associated with the mechanism of uric acid excretion seems to be indicated. In favor of this association are: the consistent occurrence of the changes in the blood lactate comparable to the consistent occurrence of the uric acid retention, the parallelism of the lactate shift with the direction of the shift in uric acid excretion, and

the fact that the shift in the blood lactate is in a direction which would be expected on the basis of the observations of Gibson and Doisy.

SUMMARY

The uric acid retention which occurs during hippuric acid synthesis from ingested sodium benzoate was investigated in physically healthy schizophrenic subjects and in two subjects with a destructive liver disease.

The degree of uric acid retention was found to be related to the amount of sodium benzoate retained in the organism. A correlation coefficient of $+ 0.79 \pm 0.044$ between the uric acid retention and the retained sodium benzoate was obtained during the third and fourth hours following ingestion of sodium benzoate.

An increase in the blood lactic acid level was observed during the synthesis. The increase in lactic acid paralleled to some degree the concomitant decrease in uric acid excretion.

Interpretations of the mechanism of the uric acid retention are presented in which the uric acid retention is considered a consequence of the absorption of benzoate rather than a phenomenon associated with the process of synthesis of hippuric acid. The changes in the blood lactic acid level are considered evidence of some involvement of lactic acid metabolism in the uric acid retention.

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IN VIVO HEMOLYSIS PRODUCED BY SOAP INJECTION¹

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Evidence indicating that hemolysis plays a rôle in the normal destruction of red blood cells has been reviewed by Isaacs (1937). The potent hemolytic substance demonstrable in the thoracic duct lymph of dogs following the ingestion of a high fat meal (Johnson and Freeman, 1938) appears to be free fatty acids or soaps which have escaped resynthesis to neutral fats (Freeman and Johnson, 1940). That the free fatty acids and soaps reach the blood stream in sufficient quantities to damage the red cells has been demonstrated in bile fistula dogs in vivo (Loewy, Marchello, Freeman and Johnson, 1943), in normal dogs (Longini, Freeman and Johnson, 1942), and in normal human beings (Johnson, Longini and Freeman, 1943). Other workers (Faust and Schminke, 1908; Meyerstein, 1912; Adler, 1913; Brinkman, 1929) have pointed out the importance of hemolysis by free fatty acids.

Since it appears that a given hemolysin is far less potent in vivo than it is when tested in vitro by the usual procedures (Ponder, Hyman and White, 1941), it was felt desirable to obtain additional data on (1) the actual quantity of free fatty acids or soaps needed to produce hemolytic action in vivo; (2) the quantitative relationships between amount of hemolysin injected and the extent of the hemolysis, and (3) the rapidity with which soap injection causes hemolysis.

METHODS. Dogs were anesthetized by the intravenous injection of 300 mgm. of barbitol sodium per kilogram of body weight. Bile pigment excretion was employed as a measure of hemolysis. The cystic ducts were tied, and the common bile ducts were cannulated for the collection of bile. The left jugular vein was cannulated for injections. In all dogs—control and experimental—a stomach tube was passed and 20 cc. of 0.5 per cent sodium glycocholate were administered every 30 minutes to insure a sustained flow of bile. In collecting bile from anesthetized dogs it is frequently observed that the volume of secretion falls off rapidly although the pigment excretion remains constant. The administration of various bile acids causes definite choleresis but does not alter the pigment excretion (Berman, Snapp, Ivy, Atkinson and Hough, 1940; Hooper, 1916).

In addition, Ringer's solution was injected into the jugular vein at the rate of 0.5 cc. per minute throughout the period of observation. In the experimental animals, varying amounts of sodium oleate (Merck) dissolved in Ringer's solution were substituted for the ordinary Ringer's solution for a period of one hour, after an initial control period of one hour. These sodium oleate-Ringer's

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solutions were turbid, indicating that the pH was lower than 8.0 and that partial or complete hydrolysis of the sodium oleate occurred (Leathes and Raper, 1925; Medveczky and Vatin, 1930). This suggests that the active principle in these preparations probably was some intermediate in the hydrolysis process or was free fatty acid. These substances are far more active at body temperature than at room temperature (Edward, 1939).

Bile samples were collected every half-hour and were analyzed for bilirubin content using the oxidation technique described by Malloy and Evelyn (1937).

TABLE 1

Hourly output of bilirubin in 21 dogs, as modified by the intravenous injection of sodium oleate
The soap was injected over a period of one hour, following the control hour

DOG NO.	WEIGHT	SOAP INJECTED	MILLIGRAMS BILIRUBIN EXCRETED PER KILOGRAM						
			Control	Hours after injection began					
				1	2	3	4	5	6
	kgm.	mgm. per kgm.							
1*	8.1	None	0.180	0.116	0.131	0.142	0.142	0.146	
2	18.9	None	0.272	0.278	0.176	0.138	0.075		
3	8.4	None	0.137	0.115	0.160	0.104	0.091	0.088	
4	13.8	None	0.179	0.221	0.184	0.125	0.101	0.116	
5	20.6	None	0.143	0.091	0.166	0.098	0.090	0.105	
6	10.9	3.6	0.473	0.439	0.491	0.479			
7*	19.5	5.0	0.174	0.256	0.171	0.169			
8	11.7	5.1	0.112	0.157	0.211	0.187	0.156	0.109	0.111
9	12.7	5.9	0.217	0.228	0.305	0.422	0.392		
10	16.5	7.5	0.239	0.250	0.355	0.432	0.390	0.366	0.397
11	10.9	9.2	0.110	0.190	0.246	0.175			
12	6.8	10.0	0.112	0.201	0.251	0.334			
13	17.3	10.0	0.139	0.214	0.200	0.250	0.205	0.205	0.210
14	4.3	12.5	0.261	0.365	0.430	0.291	0.261		
15*	13.6	15.0	0.222	0.287	0.655	0.265	0.201	0.265	
16	10.9	20.0	0.105	0.154	0.277	0.300			
17	13.6	25.0	0.133		0.378	0.304			
18	18.7	25.0	0.113	0.119	0.242	0.276	0.417	0.302	
19*	23.0	30.0	0.048	0.106	0.207	0.358	0.332	0.317	
20	12.1	41.3	0.205	0.456	0.500	1.438	0.338	0.795	
21	8.0	50.0	0.235	0.490	0.708				

* Plotted in figure 1.

Aliquot samples of 0.1 cc. were used instead of the 0.5 cc. suggested by these authors, since the latter quantity gave solutions that were too concentrated.

RESULTS. In the control dogs, the volume of bile secreted tended to remain rather constant or to decrease gradually. The quantitative bile pigment output likewise remained constant or gradually decreased (see table 1, dogs 1-5).

The injection of sodium oleate in Ringer's solution usually but not always increased the volume of bile secreted. Bile pigment excretion was increased in every animal in which more than 3.6 mgm. of soap per kilogram was injected,

and in almost all cases, an increased excretion of bile pigment commenced in the course of the hour during which the oleate was injected. The correlation between the amount of soap injected and the quantity of bile pigment excreted can be seen from the data of table 1 and is graphically illustrated in figure 1. The injection of 5 mgm. of soap (B) per kilogram body weight caused the excretion of an extra 0.074 mgm. of bilirubin per kilogram, and the injection of 15 mgm. of soap (C) per kilogram produced 0.563 extra mgm. of bilirubin per kilogram. Not all of the animals (e.g., C) were followed for a time sufficient to allow for the excretion of all of the extra bile pigment resulting from the hemolysis caused by soap injection.

Discussion. Ponder (1936) has pointed out that, although a single large injection of a lysin may fail to produce intravascular hemolysis, small quantities

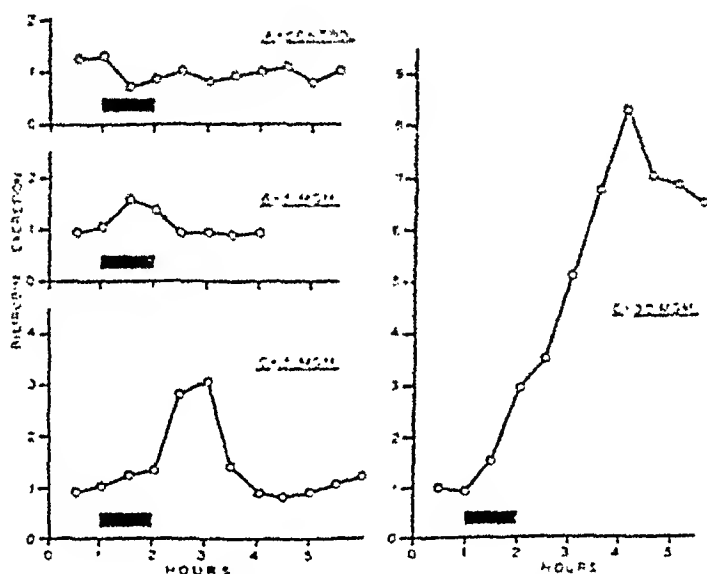


Fig. 1. Effect on bilirubin excretion of intravenous injection of Ringer's solution (A—control), 5 mgm. sodium oleate per kilogram body weight (B), 15 mgm. per kilogram (C), and 30 mgm. per kilogram (D). Units of bilirubin excretion are arbitrary, the control flow being expressed as unity.

supplied over a period of time may do so. The latter observation is borne out by our findings, since as little as 5.0 mgm. of soap per kilogram injected over a period of one hour produced measurable hemolysis.

It is of interest to attempt an estimate of the possible significance of these findings in the daily destruction of red blood cells in normal man. Does fat ingestion contribute to this process?

In dog 15 (table 1, and fig. 1C) the injection of 15 mgm. of soap per kilogram caused the excretion of an extra 0.563 mgm. of bilirubin per kilogram. Estimating 1 gram of hemoglobin to yield 34.9 mgm. of bilirubin (Cruz, Hawkins and Whipple, 1942), we conclude that, under the conditions of the experiment, 1 mgm. of injected soap liberated roughly 1 mgm. of hemoglobin.

Attempts have been made to estimate the quantity of free soap which enters

the blood stream after a fat meal. Freeman and Friedemann (1935) were able to account for only 88 per cent of the fatty acids present in chyle as triglycerides. Other investigators (Munk, 1880; Moore, 1903; Artom and Peretti, 1936) using varied techniques have shown that from 3.0 to 45.0 per cent of the fatty acids in chyle are not combined with cholesterol or glycerol. Most of these figures indicate that about 10 per cent of the fatty acids in chyle remains free. If this be true, at least 8 grams of free fatty acids or soap could enter the blood stream in a man on a diet containing 100 grams of fat. These 8 grams should free about 8 grams of hemoglobin, or roughly that contained in 50 cc. of blood. If man's blood cells survive 100 days, the cells of about 50 cc. are destroyed daily. This suggests that the fatty acids of a normal diet could account for all of the normal daily red blood cell destruction. This estimate is probably high, since in the dog experiments the soap was injected in one hour, while in man the duration of maximal absorption of digested fat lasts probably more nearly three hours, which might reduce the estimated proportion of red cells destroyed daily by fatty acid from 100 per cent to perhaps 35 per cent.

Further light is shed on the problem by the experiments of Loewy, Freeman, Marchello and Johnson (1943). This work demonstrated that adding 5 to 10 grams of fat per kilogram to the diet of dogs in chronic experiments increased red cell destruction by an average of 37 per cent. In a normal man, the ingestion of about one-fifth this amount of fat per day might be expected to account for about 8 per cent of the normal daily red cell destruction.

From these different approaches to the problem yielding figures of the same order of magnitude, it would seem conservative to estimate that from 8 per cent to 35 per cent of the daily red cell destruction occurring in a normal man on a normal diet is due to the ingestion of fat, and the absorption of lytic quantities of fatty acids and soaps. This represents a significant fraction of the normal daily red cell destruction from all causes.

This conclusion lends further support to the concept of Johnson and Freeman (1938) that absorption of the products of fat digestion into the lymphatics (Freeman, 1940) is an adaptive mechanism, which prevents excessive hemolysis partly because of the dilution of the lytic agent before it comes into contact with red blood cells.

SUMMARY AND CONCLUSIONS

1. In dogs anesthetized with barbital, the rate of red blood cell destruction was determined by collection of bile and quantitative analysis of the bile pigment excretion.

2. The intravenous injection of as little as 5.0 mgm. of soap or fatty acid per kilogram produced a definite increase in red blood cell destruction.

3. Red cell destruction was greater when larger quantities of fatty acid or soap were injected.

4. Bile pigment excretion started to increase in the course of the hour during which the fatty acid or soap was injected. In some cases the increased secretion persisted for some hours after the injection was stopped.

5. Calculations from these and other data indicate that absorbed free fatty acids and soaps from the fat of a normal diet are responsible for the lytic destruction of a significant proportion of the daily red blood cell destruction in a normal human being. Estimated conservatively, red cell destruction from this cause is from 8 per cent to 35 per cent of the total daily destruction.

6. Further support is lent to the concept that absorption of the products of fat digestion into the lymphatics is an adaptive mechanism, preventing excessive destruction of red blood cells.

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ACTION OF EQUINE GONADOTROPIN IN NORMAL AND HYPOPHYSECTOMIZED IMMATURE MALE RATS^{1, 2}

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The effectiveness of gonadotropic hormones may be markedly influenced by the frequency or route of injection. A single injection of human chorionic or anterior pituitary gonadotropin is not as effective as the administration of the same total dose in divided quantities. Equine gonadotropin (pregnant mare serum), however, has been shown to be just as effective when administered to the immature rat in a single subcutaneous injection as when the injections are divided (1-5). Pencharz (6) demonstrated in both normal and hypophysectomized female rats that the daily administration of a low dose of equine gonadotropin is more effective by the intraperitoneal than by the subcutaneous route, although Evans et al. (7) failed to find this difference using a greater dosage. A single injection of equine gonadotropin, regardless of the route of administration, exerts a somewhat greater effect on the ovaries of hypophysectomized immature rats than do daily subcutaneous injections (8). Corpora lutea formation resulted in some animals following intraperitoneal and intravenous injections of a low dose but was not observed after subcutaneous hormone administration (8).

The increase in seminal vesicle weight in the male rat has been proposed as an assay method for luteinizing hormone (9). In view of the variation in ovarian histology caused by varying the route of administration it was deemed of interest to study the response of the normal and hypophysectomized immature male rat to equine gonadotropin administered by several different injection routes.

MATERIALS AND METHODS. Normal immature male rats, 24 to 26 days of age, were used to study the influence of the route of injection. Equine gonadotropin (Gonadin)³ was injected *a*, subcutaneously; *b*, intraperitoneally in equally divided doses once daily for 5 days; *c*, subcutaneously, and *d*, intraperitoneally in a single injection.

Equine gonadotropin was administered either subcutaneously or intraperitoneally in equally divided doses once daily for 5 days to hypophysectomized immature rats. These rats were hypophysectomized at 30 to 36 days of age and a 5-day postoperative interval was allowed to elapse in all cases before treatment was instituted. All pituitary capsules were serially sectioned, stained with Mallory's triple stain and examined microscopically. Only data from completely hypophysectomized rats are presented.

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² Grateful acknowledgement is made to Mr. Jacob P. Halperin for valuable technical assistance.

³ Equine gonadotropin or pregnant mare serum, Gonadin, was generously supplied by Dr. D. H. Wonder, Cutter Laboratories, Berkeley, California.

All animals were autopsied 120 hours after the initial injection. Weights of the adrenals, testes, ventral prostates and seminal vesicles (including the coagulating gland and any contained fluid) were taken.

RESULTS. Equine gonadotropin was initially administered to normal immature male rats. Each of 3 different total dosages was tested by 4 different methods of injection. A stimulation of the seminal vesicle and ventral prostate weight was obtained with a total dosage of 1 r.u. and the increase in weight of

TABLE 1

Influence of the mode of administration on the response of the immature male rat to equine gonadotropin

NO. OF RATS	BODY WEIGHT		TREATMENT	ACCESSORY ORGAN WEIGHT (MG. $\pm E_m$)		
	Start	End		Testes	Sem. ves.	Ventral prostate
Total dosage—1 r.u.						
	gm.	gm.				
7	40.0	53.7	Daily subcutaneous $\times 5$	456	14.1 ± 1.7	51.1 ± 3.1
7*	39.6	53.9	Daily intraperitoneal $\times 5$	439	15.0 ± 0.9	49.4 ± 2.1
6	43.8	57.7	Single subcutaneous	479	13.7 ± 1.3	50.2 ± 3.7
7	39.6	51.9	Single intraperitoneal	410	12.7 ± 1.7	45.6 ± 2.7
Total dosage—2.5 r.u.						
16	42.3	57.0	Daily subcutaneous $\times 5$	468	13.4 ± 1.2	49.7 ± 7.4
13	46.2	61.6	Daily intraperitoneal $\times 5$	537	23.5 ± 1.5	46.8 ± 4.5
14	44.1	54.6	Single subcutaneous	472	14.7 ± 1.2	45.0 ± 3.6
12	43.7	59.4	Single intraperitoneal	461	13.9 ± 1.1	45.5 ± 2.3
Total dosage—5.0 r.u.						
14	44.8	56.8	Daily subcutaneous $\times 5$	482	25.9 ± 1.3	66.3 ± 1.2
10	49.2	64.0	Daily intraperitoneal $\times 5$	568	37.7 ± 2.8	78.0 ± 3.0
10	44.3	58.6	Single subcutaneous	444	21.5 ± 3.3	56.6 ± 2.7
13	46.0	54.8	Single intraperitoneal	446	25.6 ± 2.4	64.2 ± 2.9
Normal controls						
22	46.4	66.6		458	8.7 ± 0.3	33.6 ± 1.4

E_m = mean deviation of the mean.

these accessory organs was of the same degree regardless of the manner of injection. When the total dosage was increased to 2.5 r.u., the daily intraperitoneal route proved to be the more effective mode of administration. It was noted, however, that seminal vesicle weight was not increased to a greater extent with the 2.5 r.u. dose than with the 1 r.u. dose except where the daily intraperitoneal injection method was used (table 1).

Results similar to those obtained with the 2.5 r.u. dose were also obtained with a 5 r.u. total dose. The daily intraperitoneal route again proved to be the most

effective manner of administration. The single injection methods were just as effective as the daily subcutaneous route over the 120-hour test period.

Since the intraperitoneal injection method was more effective than the subcutaneous route when the hormone was administered once daily in 5 equally divided daily doses in normal rats it was deemed of interest to investigate the reaction of the hypophysectomized rat. It was found that a 2.5 r.u. total dose would definitely stimulate the accessory reproductive organs of the hypophysectomized immature male rat but the varied methods of administration exhibited no difference in potency. Greater activity of the intraperitoneal over the subcutaneous route, however, was obtained by using a 5 r.u. dosage (table 2).

Testis weight in the treated hypophysectomized rats, while greater than that of hypophysectomized controls 10 days postoperative, compared favorably with testis weights obtained 5 days postoperatively, indicating a maintenance action.

TABLE 2

Responses of hypophysectomized immature male rats to equine gonadotropin

NO. OF RATS	BODY WEIGHT AVERAGE		MODE OF INJECTION	AVERAGE ORGAN WEIGHT (MG. \pm E _m)		
	Start	End		Testes	Sem. ves.	Vent. prostate
Hypophysectomized control animals						
7	gm. 80.7	gm. 77.5	None	231	7.6 \pm 0.9	9.5 \pm 0.8
Animals receiving 0.5 r.u. daily for 5 days						
10	86.1	83.0	Subcutaneous	443	14.9 \pm 2.1	26.0 \pm 2.5
7	85.3	80.6	Intraperitoneal	543	15.7 \pm 2.0	26.4 \pm 3.3
Animals receiving 1.0 r.u. daily for 5 days						
13	82.0	76.5	Subcutaneous	335	17.9 \pm 2.9	32.0 \pm 1.4
11	87.0	81.6	Intraperitoneal	523	42.8 \pm 5.0	36.0 \pm 3.2

Secondary spermatocytes were present in the seminiferous tubules but spermatids were absent.

DISCUSSION. The daily administration of equine gonadotropin to normal and hypophysectomized immature male rats reveals the greater effectiveness of the intraperitoneal over the subcutaneous injection route as determined by seminal vesicle weight increase. Dosage was found to be a determining factor, however, since total dosages of at least 2.5 r.u. and 5 r.u. had to be injected into normal and hypophysectomized rats respectively to detect this difference.

The anterior lobe of the prostate gland of hypophysectomized male rats has been shown to be more sensitive than the seminal vesicles in response to hog pituitary extract (10). The ventral prostate of the rat responded readily to small doses of equine gonadotropin but the weight of this organ did not vary when the route of administration was altered.

The increased effectiveness of the intraperitoneal route as compared with the subcutaneous route in hypophysectomized male and female rats indicates another difference between pregnant mare serum and the anterior pituitary gonadotropic extracts. Fraenkel-Conrat et al. (11) found that the route of administration did not influence the activity of their interstitial cell stimulating hormone (ICSH) in male rats. Fevold (12) reported FSH to be ineffective intraperitoneally whereas Evans et al. (13) found FSH no more active when administered intraperitoneally than when injected subcutaneously into rats.

Previous experiments on hypophysectomized immature female rats indicated that a 10 r.u. dose of equine gonadotropin injected intraperitoneally causes the formation of corpora lutea whereas the same dosage injected subcutaneously does not (8). In fact, at least 40 r.u. of the hormone had to be injected by the subcutaneous route to cause corpora lutea formation (14). Further evidence to show that the luteinizing action of equine gonadotropin varies with the route of administration is shown in these experiments on hypophysectomized immature male rats. Using seminal vesicle weight as an index of luteinizing action, it was found that a 5 r.u. total dosage injected intraperitoneally increased seminal vesicle weight to 42.8 mgm. whereas it has been shown previously and with the same material but injected subcutaneously that a total dosage of 100 r.u. must be used to equal this response (15). Thus the luteinizing action of equine gonadotropin, under certain experimental conditions, becomes more pronounced when the hormone is administered intraperitoneally. These experiments emphasize the importance of dosage and route of administration in the response elicited by equine gonadotropin in the rat.

SUMMARY

The response of normal and hypophysectomized immature male rats to equine gonadotropin administered by varied routes was studied. A single subcutaneous or intraperitoneal injection was equally as potent as were divided subcutaneous injections. The greatest increase in seminal vesicle weight was produced by daily intraperitoneal injections.

These data show that the route of administration is a determining factor in the luteinizing action of equine gonadotropin.

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SENSITIVITY OF DROSOPHILA TO POISONING BY OXYGEN¹

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The range of oxygen tensions within which aerobic life is possible is characterized by upper as well as lower limits. The existence of upper limits results from the toxicity of high tensions of oxygen. An examination of the extensive literature in regard to this phenomenon reveals that a sensitivity to poisoning by oxygen apparently obtains for the entire taxonomic spectrum, from bacteria (Thaysen, 1934; Bean, 1941) and protozoa (Cleveland, 1925; 1934) up through the human species (Case and Haldane, 1911). Since anaerobic organisms are especially sensitive to the presence of oxygen (Quastel and Stephenson, 1926), the deleterious properties of oxygen excess are even more universal than of oxygen deficiency. Thus consideration of comparative physiology suggests the possibility that high tensions of oxygen affect systems that are of general distribution among organisms.

The object of the present report is to describe the sensitivity of a single species of animal to poisoning by oxygen. Since insects appeared to be strategically suited for the study, an inbred strain of *Drosophila* was used as an experimental animal. The reasons for this choice may be summarized as follows: 1, genetically homogeneous strains were available that essentially eliminated variability due to hereditary factors; 2, the small size of insects permits the use of high pressures within glass chambers without serious risk of explosion; 3, the nature of the respiratory system facilitates rapid equilibration, *via* the tracheal system, with experimental gas mixtures; 4, insects are insensitive to abrupt changes in pressure; and 5, the degree of recovery following non-lethal poisoning was measurable in terms of the frequency of wing-beat during flight.

METHODS. All the experiments described in this report were performed on an inbred strain of *Drosophila azteca* var. Deer Creek, that was provided by Prof. Th. Dobzhansky. The insects were reared and isolated in bottles containing a standard agar-cornmeal-molasses-yeast preparation at a temperature of $20.0 \pm 0.5^\circ\text{C}$. The age of the insects used in experiments was controlled within ± 0.5 day.

This particular strain of *Drosophila* was chosen as a test animal because a previous study of the frequency of wing-beat of 24 species and varieties had demonstrated a minimal variability in the case of "Deer Creek" (Reed, Williams and Chadwick, 1942).

Two techniques were used to study the sensitivity of *Drosophila* to poisoning by oxygen. In the first group of experiments we measured the lengths of time necessary for specific high tensions of oxygen to kill the insects during the period

¹ This study was aided by a grant from the Josiah Macy, Jr. Foundation.

of exposure. This was accomplished by placing the animals within vials, loosely plugged with cotton, and sealing them within steel pressure tanks (size D, with screw valve head). Oxygen was then added from a commercial cylinder and the experimental oxygen tension ascertained at room temperature by means of test gauges fitted to a needle valve, the fifth of an atmosphere of oxygen initially present in the air-filled chamber being taken into account. Thereupon, the tanks were immersed in water baths at temperatures controlled within $\pm 0.05^{\circ}\text{C}$.

Approximately ten males and ten females of each of four ages were tested in each tank. These age groups were 0-1, 3-4, 10-11, and 20-21 days old, thus covering the major portion of the adult life of *Drosophila azteca*. By preparing a series of tanks and opening them after varying periods of time, it was possible to ascertain within progressively narrower limits the duration of exposure necessary for lethal effect in the presence of specific combinations of temperature and oxygen tension. Since the extremes of variability are not likely to occur within samples as small as twenty animals, the minimal duration of lethal exposure was judged in terms of the absence of any recovery (100 per cent kill). The true lethal duration of exposure was considered to lie midway between the minimal lethal exposure and the maximal non-lethal exposure.

In a second group of experiments the toxicity of standard high tensions of oxygen was determined in terms of the exposure necessary to render *Drosophila* incapable of any spontaneous or reflex movements. This was possible by means of the apparatus previously used in connection with studies of insect flight and described by Williams and Chadwick (1943). The Pyrex chamber was washed out with oxygen and nine additional atmospheres of oxygen rapidly added, the apparatus being immersed in a water bath at a temperature of $20.0 \pm 0.05^{\circ}\text{C}$. Stroboscopic measurements of the frequency of wing-beat were performed at one atmosphere of pressure before and after exposure to oxygen in order to ascertain the degree of recovery.

Stages in oxygen poisoning and degrees of recovery. It has long been recognized that the convulsions so characteristic of birds and mammals during the final stage of oxygen poisoning are generally absent in lower organisms. *Drosophila* during the period of non-lethal exposure to high oxygen tensions nevertheless exhibits characteristic symptoms that culminate in the loss of spontaneous and reflex excitability.

The several stages that progressively appear may be summarized as follows: 1, an initial period of excitation accompanied by vigorous leg movements; 2, loss of the tarsal reflex controlling the extension and flexion of the wings and, at lower total pressures, the initiation of flight; 3, spontaneous movements of the legs gradually decrease in frequency and the animal stands motionless on the platform; 4, a tendency appears for the legs to become stiff and flexed and ultimately the stance on the platform is lost; 5, loss of spontaneous movements; 6, loss of reflex excitability.

The extent to which an animal recovers after exposure to high oxygen tensions is determined by the degree of poisoning that has ensued. Brief exposure to high oxygen tension produces effects that are largely reversible. For example,

ten female *Drosophila*, 10–11 days old, were placed in each of two pressure tanks and exposed to 5 atmospheres of oxygen, at 20°C., for 1 and 2 hours, respectively. At the end of this treatment the insects were stored in food bottles and 24 hours later their wing-beat frequencies determined. As shown in table 1, the animals exposed to oxygen for 1 hour possessed wing-beat frequencies essentially identical with those of untreated controls. A slight but significant decrease characterized the group exposed to oxygen for 2 hours.

Similarly, only small residual effects were found for animals poisoned with 10 atmospheres of oxygen until reflex activity was lost. When these individuals were immediately decompressed and placed in air, flight ability was rapidly regained. For example, a group of nine 10–11 day old animals had an average wing-beat frequency of 204 ± 1.9 double-beats per second before treatment and a subsequent frequency of 198 ± 1.9 after 3 hours of recovery.

Longer periods of exposure were followed, upon decompression and storage in air, by progressively lesser degrees of recovery. The nervous system is apparently a site of primary injury, for the first powers to be permanently lost are those

TABLE 1

Wing-beat frequencies of Drosophila after 24 hours of recovery following non-lethal oxygen poisoning by 5 atmospheres of oxygen

DURATION OF EXPOSURE	NUMBER OF ANIMALS	AVERAGE WING-BEAT FREQUENCY	DIFFERENCE
<i>hours</i>		<i>(double-beats/sec.)</i>	
0 (Control)	5	201 ± 2.5	
1	10	200 ± 1.1	-1 (0.5%)
2	10	192 ± 1.3	-9 (4.5%)

concerned with the maintenance of balance and equilibrium. After still longer exposures, stiff tetanic leg movements are the only indication of recovery, and ultimately the animals are killed during the period of exposure.

Relation between oxygen tension and toxicity. There is general agreement in the literature that the toxic action of any given high tension of oxygen is a function of the partial pressure of oxygen and is essentially independent of the tensions of inert gases, such as nitrogen, that may be simultaneously present. Our experiments on *Drosophila* have confirmed this fact. For example, at 20°C. the lethal duration of exposure to 5 atmospheres of oxygen was 6.75 hours, and to 25 atmospheres of carbon dioxide-free air, 6.50 hours (animals 10–11 days old). It is therefore apparent that high tensions of nitrogen have no effect in protecting the tissues from the toxic action of high tensions of oxygen.

The degree to which the toxicity of oxygen increases with oxygen tension was studied in terms of the length of time necessary for a series of oxygen tensions to have lethal effect. The results obtained at 20°C. on a total of 300 animals (10–11 days old) are recorded in figure 1. Marked increase in toxicity clearly accompanies progressively higher oxygen tensions. The rate of poisoning (reciprocal

of time) by 10 atmospheres of oxygen was approximately eight times that by 2 atmospheres of oxygen.

As demonstrated in figure 2 (upper curve), this relationship between oxygen tension and toxicity is linear on double logarithmic co-ordinates, conforming to the equation:

$$y = 2.08x^{-1.81}$$

Where:

y = lethal duration of exposure in hours.

x = oxygen tension in atmospheres.

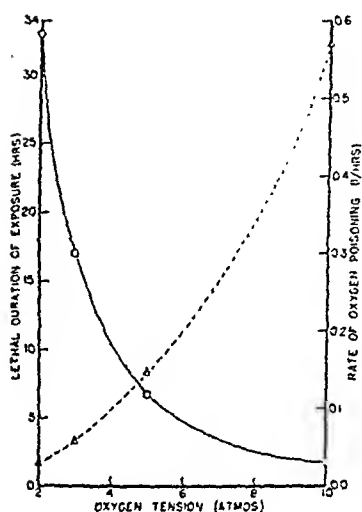


Fig. 1

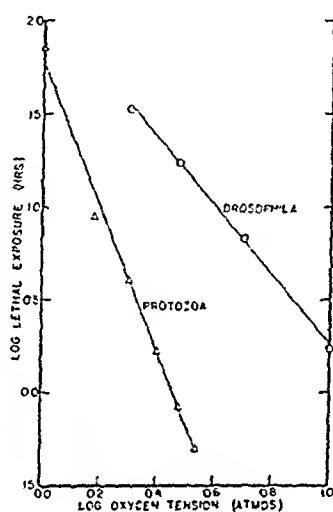


Fig. 2

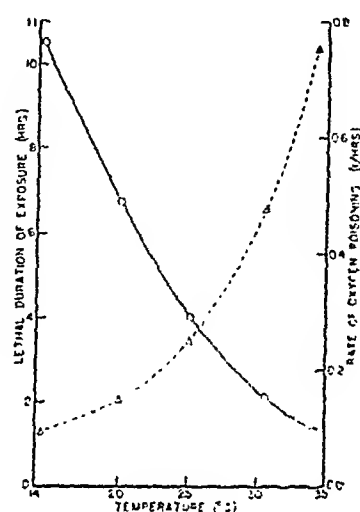


Figure 3

Fig. 1. Toxicity of oxygen as a function of oxygen tension. Circular points, lethal duration of exposure; triangular points, rate of oxygen poisoning. *Drosophila* 10 to 11 days old at 20°C.

Fig. 2. Log oxygen toxicity as a function of log oxygen tension, for *Drosophila* and for the symbiotic protozoa of termites.

Fig. 3. Toxicity of 5 atmospheres of oxygen as a function of temperature. Circular points, lethal duration of exposure; triangular points, rate of oxygen poisoning. *Drosophila* 10 to 11 days old.

The only data on other animals that are sufficiently extensive for comparison are the measurements noted by Cleveland (1925; 1934) in regard to the toxicity of oxygen for the intestinal protozoa of termites. It is noteworthy that the lethal durations of exposure listed by Cleveland likewise agree with an equation of the double logarithmic type (lower curve in fig. 2); namely:

$$y = 1.73x^{-3.80}$$

Thus at least for *Drosophila* and for these protozoa the toxicity of oxygen as a function of oxygen tension conforms to the relationship that generally describes time-concentration curves in pharmacology (Clark, 1933).

A comparison of the curves in figure 2 is of interest since Cleveland's data were obtained in the course of defaunation experiments in which the symbiotic proto-

zoa were poisoned by oxygen without injury to the host termite. If the sensitivity of the termite agrees roughly with that of *Drosophila*, then it is apparent why defaunation is possible: whereas the toxicity of oxygen for *Drosophila* varies as approximately the second power of oxygen tension, for the protozoa the toxicity varies as approximately the fourth power. Thus, as demonstrated in figure 2, with an increase in oxygen tension the duration of exposure necessary for lethal effect becomes progressively shorter for the protozoa relative to the insect.

If the toxicity of oxygen for other animals likewise conforms to equations of the double logarithmic type, then it may be possible to compare under standardized conditions the sensitivities of other organisms in terms of the constants k and n . Such a comparison should reveal the utility of oxygen poisoning as a technique in therapeutics.

Relation between temperature and oxygen toxicity. Since the vast majority of previous studies of oxygen poisoning were performed on mammals, the relation between body temperature and the toxicity of oxygen has been generally disregarded. It is nevertheless important to know whether a rise in body temperature, as in fever, facilitates oxygen poisoning; such information is likewise necessary to compare the sensitivities of cold-blooded animals, which have in most instances been studied with temperature uncontrolled.

At an oxygen tension of 5 atmospheres the duration of exposure necessary for lethal effect was studied on a total of 400 animals (10–11 days old) over a temperature range from 14.4 to 34.2°C. The results described in figure 3 indicate that the toxicity increased greatly with a rise in temperature: at 34.2°C. the rate of oxygen poisoning was nearly eight times as rapid as at 14.4°C.

It is probable that this action of temperature is a general property of poisoning by high tension of oxygen, for similar observations have been reported for protozoa (Cleveland, 1934), bacteria (Thaysen, 1934), and mammals (Campbell, 1937a, b). Hence the effect of temperature on the toxicity of high tensions of oxygen is contrary to its effect on the solubility of oxygen in body fluids, the former varying directly and the latter inversely with temperature.

Since in poikilothermic animals such as *Drosophila* metabolic rate is a function of environmental temperature, it is a reasonable deduction that the relation between oxygen toxicity and temperature is, at least in part, a relation between oxygen toxicity and metabolic rate. This inference agrees with the results reported by Campbell (1937b), who found that the toxicity of oxygen for white rats was enhanced by thyroxin and adrenalin, and retarded by thyroidectomy and starvation.

Effect of age of animal. Several investigators have noted that old animals are more easily poisoned by oxygen than young animals. Faulkner and Binger (1927) observed that old turtles are more susceptible to oxygen poisoning than young turtles. Similarly, Smith *et al.* (1932a, b) report such an increase with age in the sensitivity of white rats to chronic oxygen poisoning, an effect attributed primarily to a simultaneous and progressive decrease with age in the thickness of the respiratory epithelium of the lung.

In order to ascertain whether this effect of age likewise obtains in the case of

insects, where lungs are absent and where the respiratory and circulatory systems are organized on a wholly different plan from those of vertebrates, we studied the relative sensitivities of four different age groups of *Drosophila* to poisoning by 10 atmospheres of oxygen, at 20°C. These experiments were performed on single individuals within the flight chamber, the duration of exposure necessary for complete loss of spontaneous and reflex movements being measured. A total of 64 animals was tested.

As indicated in figure 4, the critical duration of exposure was longer for young than for old animals; the rate of poisoning varied in a linear fashion with age, the differences between all of these points being statistically significant.

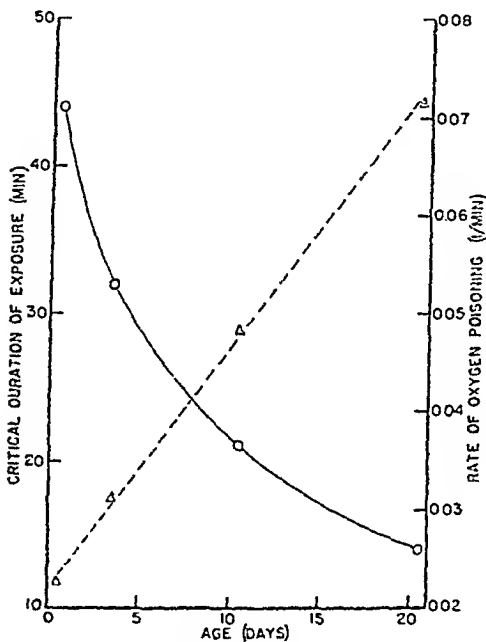


Fig. 4

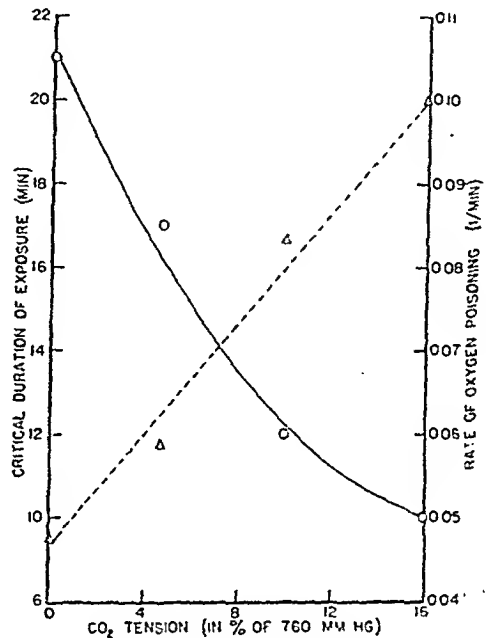


Fig. 5

Fig. 4. Toxicity of 10 atmospheres of oxygen as a function of the age of *Drosophila*. Circular points, critical duration of exposure; triangular points, rate of oxygen poisoning. At 20°C.

Fig. 5. Toxicity of 10 atmospheres of oxygen as a function of environmental carbon dioxide tension. Circular points, critical duration of exposure; triangular points, rate of oxygen poisoning. *Drosophila* 10 to 11 days old at 20°C.

It is a curious fact that this effect of the animal's age in determining the duration of exposure necessary for the loss of reflex activity was much less pronounced when the toxicity of oxygen was judged in terms of the time necessary for lethal effect. Nevertheless, very young animals were clearly more resistant than very old animals.

Thus the effect of age in modifying sensitivity to oxygen poisoning obtains not only for animals that possess lungs, but also for insects in which lungs are absent.

Effect of carbon dioxide. Since the pioneering researches of Bert (1878), it has been generally recognized that mammals are more rapidly poisoned by high tensions of oxygen when the carbon dioxide tension of the atmosphere is in-

creased (Bean, 1931; Shaw *et al.*, 1934). This observation is of interest in view of the theory advanced by Gesell (1923); oxygen poisoning was considered to result from an accumulation of carbon dioxide in the tissues, consequent to the failure of reduction of oxyhemoglobin in the presence of high concentrations of oxygen in physical solution in the blood.

Such a mechanism may very possibly play a significant rôle in the poisoning of vertebrates by oxygen. Yet from a standpoint of comparative physiology it is important to note that the sensitivity of organisms to oxygen poisoning is not reserved to those animals that possess respiratory blood pigments and the possibility of "Bohr effects." Many of these lower organisms, such as certain protozoa (Cleveland, 1925; 1934) and certain insects (Williams, unpublished data), are more sensitive than mammals to high tensions of oxygen. Furthermore, it is known from experiments to be considered elsewhere that *Drosophila* can sustain prolonged exposure to tensions of carbon dioxide as high as 15 per cent of an atmosphere (in air). An accumulation of a lethal concentration of carbon dioxide in the tissues as a result of exposure to high oxygen tensions therefore seems unlikely, especially in view of the nature of the tracheal respiratory system of insects.

Although carbon dioxide accumulation appears to be ruled out as the causal mechanism in the poisoning of insects by oxygen, it is nevertheless notable that the toxic properties of a gas mixture containing a given high tension of oxygen are increased by the addition of carbon dioxide. As shown in figure 5 the critical duration of exposure necessary to produce total loss of spontaneous and reflex activity by 10 atmospheres of oxygen decreased regularly as the carbon dioxide tension increased; the rate of poisoning can be described as a linear function of carbon dioxide tension. A total of 53 animals (10-11 days old) were tested.

Hence for insects as well as mammals, poisoning in the presence of high oxygen tensions is facilitated by carbon dioxide. The extent to which this effect may be an addition or a true potentiation is unknown.

SUMMARY

The sensitivity of an inbred strain of *Drosophila* to poisoning by oxygen was studied under a variety of experimental conditions. Two end-points were used to measure this sensitivity. In one group of experiments the length of time necessary for lethal effect was ascertained; in a second group the toxicity was judged in terms of the duration of exposure necessary for the loss of spontaneous and reflex movements. The degree of recovery following non-lethal poisoning was determined by comparing the frequencies of wing-beat during flight before and after treatment.

High tensions of nitrogen were found to be without effect in protecting the tissues against the toxic effects of high tensions of oxygen.

Marked increase in toxicity accompanies increase in oxygen tension. Under standardized conditions the rate of oxygen poisoning by 10 atmospheres of oxygen was approximately eight times that by 2 atmospheres of oxygen.

The relationship between oxygen tension and toxicity is linear on double

logarithmic co-ordinates and consequently agrees with the usual form of time-concentration curves in pharmacology. The toxicity varied as approximately the second power of oxygen tension.

A similar type of relationship obtains for the symbiotic protozoa of termites, studied by Cleveland. For the protozoan, the toxicity varies as approximately the fourth power of oxygen tension. These results suggest a general method for comparing in quantitative terms and under standardized conditions the sensitivities of other animals to oxygen poisoning.

The toxicity of high oxygen tensions increases regularly with the temperature and presumably the metabolic rate of the tissues. At 34.2°C. the rate of poisoning by 5 atmospheres of oxygen was nearly eight times as rapid as at 14.4°C.

Evidence was found that young animals are more resistant to oxygen poisoning than old animals.

The significance of carbon dioxide as the causal mechanism in oxygen poisoning is discussed. The evidence does not support a general explanation of oxygen poisoning in terms of an accumulation of carbon dioxide in the tissues, although such a process may be significant in animals containing hemoglobin.

The toxic properties of gas mixtures containing high tensions of oxygen are enhanced as the carbon dioxide tension increases.

The toxicity of oxygen, as judged from these experiments on *Drosophila*, is therefore not solely a function of oxygen tension. Among numerous other possible factors, the effect also depends on the temperature, carbon dioxide tension, and the age of the animal, all of which are known to affect metabolism. Thus oxygen poisoning must be considered not only in terms of the reagent, the absolute tension of oxygen, but also in terms of the reactant, the biochemical metabolic processes in the tissues.

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SPECIFIC GRAVITY AND TOTAL NITROGEN OF PANCREATIC JUICE SECRETED IN RESPONSE TO VARIOUS STIMULI

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The concentration of organic matter in the pancreatic juice is variable and is influenced by the type of food used to stimulate secretion (Walther, 1897; Babkin and Sawitsch, 1908a; Babkin, 1928). This conclusion is based for the most part on experiments in which the food was given by mouth or placed in the stomach through a gastric fistula. Relatively few studies have been made in which the stimulus (excepting HCl) has been placed directly in the intestine. Since most pancreatic stimuli act in the intestine and the intestinal contents following a meal of any sort are of complex and uncertain composition it is obviously impossible by feeding experiments alone to determine the specific stimuli responsible for the peculiarities of the pancreatic juice. We have undertaken to obtain more precise information by studying some of the properties of pancreatic juice secreted in response to measured quantities of specific stimulating substances placed in the intestines of fasting animals.

METHODS. The experiments were performed on unanesthetized dogs in which gastric and duodenal fistulas had been previously established under anesthesia and with aseptic precautions. The duodenal fistula was placed opposite the entrance of the main pancreatic duct. Pancreatic juice was collected through a small rubber funnel which was inserted via the duodenal fistula tube. Details of this method of collecting pancreatic juice have been reported previously (Thomas and Crider, 1940; Thomas, 1941).

The following stimuli were used in separate experiments: 10 cc. of N/10 HCl; 10 cc. of 2 per cent soap solution; 20 cc. of 5 per cent peptone solution. Each of the foregoing was injected into the intestine below the duodenal fistula. In addition some experiments were performed in which commercial secretin (4 units of "Pancreatest") was administered intravenously. The soap solutions were prepared by dissolving either "Ivory Snow" or C.P. sodium oleate in N/100 HCl. The pH of the resulting solution was near 9.5. There was little or no difference in the secretion obtained with the two preparations of soap. The peptone solution used in a majority of the experiments was prepared by dissolving "Bacto Protone" in 0.6 per cent NaCl solution. Other commercial peptones, also casein digests prepared in the laboratory, were used but without significant difference in the results.

Successive injections of the stimulating substance were made every 12 to 15 minutes throughout the experiment and the secretion was collected for a uniform period of 10 minutes beginning as soon as convenient following each injection. The sample collected during the 10 minute period following the first injection often differed markedly in its properties from the succeeding samples, doubtless

because it contained a variable amount of secretion already present in the ducts and not elaborated in response to the specific stimulus. It was therefore uniformly discarded.

The specific gravity of each sample was determined using a 1 cc. specific gravity bottle. Total nitrogen was determined on pooled samples by the micro-Kjeldahl method in about half the experiments. Results were obtained on a total of 12 dogs, but not every stimulus was used in every animal (see table 1).

RESULTS. We found that each stimulus used produced a distinctive type of pancreatic juice, easily distinguished by its specific gravity and total nitrogen content from that produced in response to any of the other stimuli, except that the secretion obtained following secretin injection could not be distinguished from that following acid stimulation.

TABLE 1

Specific gravity and total nitrogen of pancreatic juice obtained by means of various stimuli

DOG	20 CC. 5% PEPTONE			10 CC. 2% SOAP SOL.			10 CC. N/10 HCl			4 UNITS "PANCREOTEST"		
	Number of samples	Sp.g. average	Total N average	Number of samples	Sp.g. average	Total N average	Number of samples	Sp.g. average	Total N average	Number of samples	Sp.g. average	Total N average
			mgm./cc.			mgm./cc.			mgm./cc.			mgm./cc.
3-39	57	1.0309					4	1.0119		6	1.0122	
2-40	83	1.0199					8	1.0104		4	1.0111	
3-41	92	1.0163					6	1.0099		4	1.0109	
4-42	9	1.0162					3	1.0107				
5-42	9	1.0160					2	1.0098				
6-42	3	1.0247					2	1.0115				
7-42	3	1.0234					2	1.0111				
8-42	10	1.0194	7.00	10	1.0139	3.08	13	1.0095	1.33	4	1.0092	0.87
9-42	11	1.0190	6.70	11	1.0124	2.57	11	1.0104	1.54	10	1.0106	1.12
10-42	11	1.0220	8.37	12	1.0125	2.74	11	1.0093	0.98			
11-42	18	1.0182	6.40	3	1.0119	2.37	10	1.0096	0.96	3	1.0100	1.07
4-43	10	1.0236	9.36	17	1.0160	4.97	7	1.0088	0.70			

The specific gravity of the secretion obtained in response to acid or secretin stimulation ranged from 1.0084 to 1.0132 except for a single sample obtained following "Pancreotest" injection which had a sp.g. of 1.016. The specific gravity of a majority of the samples was between 1.009 and 1.011. The average for HCl was 1.0102 (twelve dogs) and for "Pancreotest" 1.0106 (six dogs). Nitrogen values ranged from 0.7 to 1.8 mgm. per cc., averaging 1.1 mgm. after HCl (five dogs) and 1.02 mgm. after secretin (three dogs).

The specific gravity of the secretion obtained in response to soap stimulation in five dogs ranged from 1.0114 to 1.0188. In a majority of the samples it fell between 1.012 and 1.016 and the general average was 1.0133. Total nitrogens ranged between 2.35 and 6.16 mgm. per cc. but only two of the pooled samples had more than 4 mgm. The average value for the five animals was 3.14 mgm.

The most concentrated secretion was obtained following the administration of

peptone. Specific gravities ranged from 1.0121 to 1.0402 in twelve dogs but only a few samples had specific gravities below 1.016 and except for those from one animal (no. 3-39) not many were above 1.025; the general average was 1.0208. Nitrogen values in samples from five dogs ranged from 6.7 to 9.36 mgm. per cc., averaging 7.56 mgm.

Considerable individual variation was noted within the group of animals studied with respect to the concentration of their pancreatic juice. This was particularly noticeable in the pancreatic juice obtained after soap or peptone stimulation and was less pronounced when secretin or HCl was used as a stimulus. One animal in particular (3-39) regularly secreted very concentrated juice in response to peptone. Soap was not tried in this dog. Another (4-43) regularly secreted a more concentrated juice in response to soap than any of the others in which soap was used. In spite of these variations the most concentrated pancreatic juice obtained from any one animal was always obtained in response to peptone stimulation and the least concentrated in response to secretin or HCl. The results on individual animals are presented in table 1.

The observed variations in concentration were not due to variations in the rate of secretion. Although the average rate of secretion following the administration of peptone was less than that following acid, in the amounts used, there were numerous instances in which acid caused less or peptone more than the usual amount of secretion, resulting in 10-minute samples of approximately equal volume. The characteristic difference in concentration was evident in these samples as in all others. Likewise, although the rate of secretion following administration of soap was approximately the same as that following acid, the secretion evoked by soap was consistently more concentrated.

Discussion. The results of previous work, confirmed by this study, prove that the pancreas responds differently to different stimuli. The present study in particular shows that this specificity of response is still manifest when the stimuli are confined to the intestine and are not complicated by the manifold stimulations associated with eating and digesting food. Clearly the various types of secretion cannot all be produced by the action of a single hormone. Either there is more than one pancreatic hormone elaborated in the intestine or some other mechanism is involved in stimulation of the pancreas by various food products present in the intestine. Probably some of the different types of secretion result from selective excitation of secretory reflexes.

It is uncertain how much significance should be attributed to the fact that the secretion obtained in response to "Pancreotest" closely resembles that produced by acid stimulation. This result certainly suggests that HCl acts solely by liberating secretin and that its action is not complicated by concomitant stimulation of secretory reflexes. On the other hand, Bylina (1913) reported that the digestive power of pancreatic juice secreted in response to HCl was reduced following administration of atropine. Babkin and Sawitsch (1908b) found that the secretin preparation which they used caused a more concentrated pancreatic secretion than did HCl and we have often noted that crude secretin causes a more concentrated secretion than relatively pure secretin. "Pancreotest" is not pure

secretin; hence the secretion which it causes may be more concentrated than would result from administration of pure secretin, and its similarity to "HCl juice" may be mere coincidence.¹

SUMMARY AND CONCLUSION

The specific gravity and total nitrogen of dog's pancreatic juice vary with the type of stimulus used to provoke secretion. Peptone in the intestine causes the most concentrated secretion, soap causes a less concentrated secretion, and HCl the most dilute secretion. The secretion caused by intravenous administration of "Pancreatost" resembles that caused by HCl in the intestine.

Acknowledgment. We gratefully acknowledge our indebtedness to Elizabeth K. Tillson for making the numerous nitrogen determinations.

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¹ Since the above was written, the work of Harper and Raper (J. Physiol. 102: 115, 1943) has come to our attention. They describe the separation from crude secretin of a substance which apparently stimulates the pancreas to secrete enzymes.

THE TESTING OF COLOR VISION IN RELATION TO VITAMIN A ADMINISTRATION

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The testing of color vision by means of the pseudo-isochromatic plates of Ishihara, Stilling and others has been accepted as the most useful measure of color blindness by the medical examining officers of the armed forces. Our observations, however, have been that many individuals who "fail" these tests can distinguish wave lengths normally and we have also observed that two complete dichromats do no worse with the test than certain individuals whose wave length discrimination is normal.

Dunlap (1) has observed that a group of individuals could improve their "color vision" by taking large amounts of vitamin A. It is a well known fact that the "color blind" can be made "normal" by observing the pseudo-isochromatic plates through rose colored glass and that the normal sees the "color blind" figures when he looks through blue glass. Such a procedure could never affect wave length discriminations. It only affects the relative intensity of various wave lengths at the eye. That vitamin therapy may effect retinal pigmentation and enable the subject to pass the test, as he can do with rose colored glasses, is not impossible. If so it would do so without improving his wave length discrimination.

These considerations led to a desire to investigate the following questions: 1. What correlation is there between the ability to discriminate hue using controlled spectral stimuli and the ability to pass the Ishihara test? 2. What correlation is there between either of these abilities and vitamin A administration or blood vitamin A? 3. What explanation can be offered for failure to pass the Ishihara test in individuals who have normal hue discrimination?

Six Ishihara "color blind" students were given 50,000 units of vitamin A² per day for eight weeks. Seven others were given the drug for 4 weeks. It was withheld during the second 4 weeks and administered again during the third period of 4 weeks. Color vision, blood vitamin A, by the method of Yudkin (2) and dark adaptation, by the method of Hecht (3) were tested in all individuals before the experimental period and again at the end of 4, 8 and 12 weeks.

The color vision was tested by the Ishihara method, by the Holmgren method and by the determination of the wave length discrimination threshold using spectral stimuli. The apparatus for this last test has been described by Townes (4) and consists of a spectrometer in which are seen two fields in juxtaposition, each illuminated with spectral light whose wave length and brightness could be independently varied. The usual precautions were taken to be sure that the

¹ Surgeon, United States Public Health Service (Bethesda, Md.).

² Afaxin in oil, 1600 units per drop, was furnished by Winthrop Chemical Co., Inc.

eye was not fatigued, and that the judgments were unequivocal. All color vision tests were carried out in a well lighted room, there being no dark adaptation. Thresholds were determined at 600, 580, 560 and 490 m μ . The extreme red end of the spectrum was determined for each individual.

RESULTS. *Vitamin A.* The figures, varying between 118 and 184 International Units per 100 cc. of blood were within normal limits (average 158). Administration of vitamin A raised the average blood concentration to 217 at the end of 4 weeks. The groups that continued the administration had a blood level of 230 at the end of 8 weeks and the other group a blood level of 175.

Dark adaptation tests gave results within normal limits. The whole group could see the red end of the spectrum normally. There was no shortening and hence no tendency toward red weakness in any of the group.

The Holmgren test showed errors which seemed to be errors in judgment rather than in vision. Some individuals would see enough greenness or redness in grays or browns to cause them to sort the wools differently than the key indicated. The dichromats (two individuals) were the only men who confused definite reds and greens. The performance of the other men who failed the Ishihara test was not worse than that of ten Ishihara normals.

In a great many cases there was improvement in the Holmgren scores on successive trials. This seemed to be due to learning the peculiarities of the test and occurred equally in the group which did not have the vitamin during the record period and whose blood vitamin values were decreasing.

Hue discrimination tests showed that two were dichromatic and were able to match wave lengths as short as 525 to 530 m μ with the red end of the spectrum. One of these is not included above because he did not take the vitamin.

Previous work has shown that the blue sensation is not stimulated by wave lengths longer than 517 to 530 m μ and that the apparent unsaturation of the yellowish parts of the spectrum is not due to trichromatic stimulation (5, 6). In the dichromat all vision above 517 to 530 m μ is served by a single sensation and there is no hue discrimination in this part of the spectrum. Dichromatic vision was in no way affected by vitamin ingestion. The Ishihara test gave a correct diagnosis since the cases were classified as green blind and by examination with spectral light they were dichromats with a long spectrum.

Twelve of the "Ishihara blind" students were trichromats and could discriminate colors throughout the spectrum. About half of these men had thresholds close to the average of the population (4) and half had somewhat higher thresholds in the orange to green colors. They all had normal thresholds in the blue green (490 m μ). The data are plotted in figure 1.

Except for small fluctuating changes, all students but one showed no consistent improvement as the tests were repeated. This man (R) had the highest threshold (17 m μ at 600) in the first test and was well within the normal range in his later tests. Coincident with this improvement in hue discrimination was a shift from "total red blindness" to "partial red green blindness" on the Ishihara test, but no significant change either in the blood vitamin, the adaptometer test or in performance with the Holmgren wools.

The Ishihara test classified the 12 trichromats into 1, the "partially red green blind" (8 tests); 2, the totally "red blind" (3 tests) and the totally "green blind" (2 tests). R. changed his classification and is here counted twice. At the time of the "red blind" tests all of the subjects showed hue discrimination thresholds that were definitely higher than normal. One of the "green blind" students had very high thresholds and one normal. About half of the "partially red green blind" had high thresholds and the rest had normal thresholds.

Aside from the subject R discussed above, one other subject showed a change in vision which might be attributed to vitamin administration. He was "partially red green blind" with normal hue discrimination and a blood vitamin A of 158. After vitamin administration he ran two normal tests when his blood vitamin was high (214). When he had stopped taking the vitamin A and his

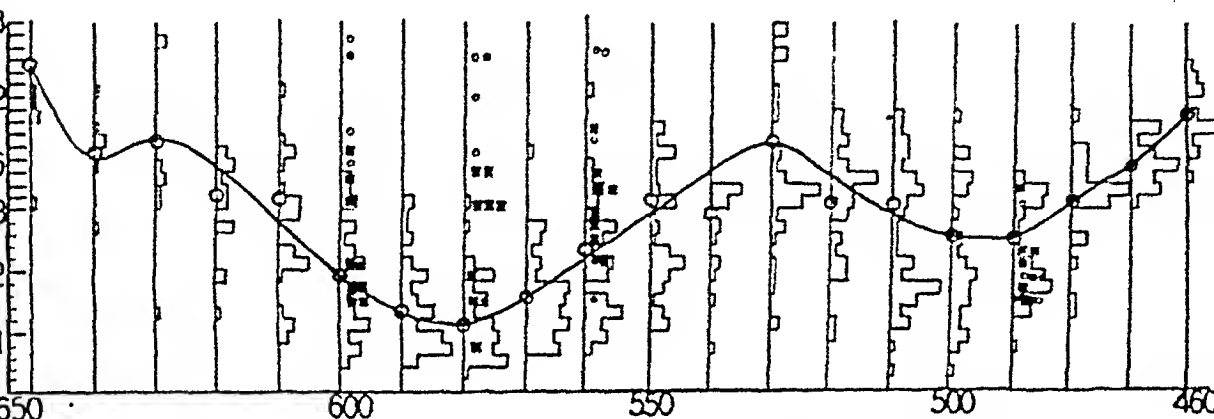


Fig. 1. Relation of wave length discrimination in the "Ishihara color blind" to that in the general population. The data of Townes (4) are plotted as a frequency histogram based on the various wave lengths tested. The smallest rectangles shown represent a single observation. The mean of Townes' data is indicated by large circles connected by a heavy line. The thresholds presented here are those of students classified as red blind, open circles; green blind, filled circles; and partial red green blind, squares.

Ordinates, thresholds in $m\mu$; abscissa, spectrum in $m\mu$.

blood vitamin was presumably back to the original figure the test was again "partial red green blind." This one observation is consistent with the notion that retinal pigmentation may have changed under the influence of vitamin therapy so as to change the Ishihara test in the same way as looking through rose colored glass. The fact that only one out of 13 individuals showed this "improvement" and the fact that hue discrimination did not improve coincidentally makes the observation of merely speculative interest.

DISCUSSION. Townes has shown that in the red to green parts of the spectrum the thresholds of hue discrimination are distributed according to simple population curves (see fig. 1). In the blue green the distribution is possibly bimodal. The color mixture data of Hamilton and Freeman are also distributed in a random fashion over the population. Townes recognizes that subjects who make anomalous mixtures of red and green to match yellow (Rayleigh equation) may

have higher wave length thresholds than those who make the usual matches. This is a tendency without great predictive value since many who make anomalous mixtures have low thresholds.

The above data lead to the conclusion that use of the Ishihara test causes the armed forces to discard a large group of candidates, about half of whom have normal wave length discrimination. The question remains however: Why do individuals consistently read the charts "wrong" when they are capable of discriminating colors that are different by the smallest nuance perceptible to the normal eye?

In charts 6, 7, 8 and 9 most of us see a reddish figure on a greenish background or a greenish figure on a reddish background. The background itself shows a pattern of intensity difference over all. When the dots of the figure are pointed out to the dichromat he cannot say that they differ in color from the dots of the background. The trichromat, who fails the Ishihara test can name correctly the color of each dot making up both figure and background, but when he looks at the card as a whole the dots whose color he can see very clearly fail to arrange themselves into a figure. He talks about a cloud of greenish dots on a reddish background or vice versa. He fails to build up a pattern in his mind out of a color difference that he can clearly see while the man who passes the test organizes the dots, whose color he recognizes no more clearly, into figure and background. The difference between the two seems to be psychic rather than sensory. The same thing applies even more strikingly to plates 12 and 13. Even the complete dichromat can recognize chroma in the scarlet and crimson dots when he looks at them individually and he can tell that they differ from the gray dots which make up the background. The complete dichromats which we have tested and some of the trichromats with normal wave length discrimination fail to organize these colored dots into figures and can only see a "cloud of red dots." Some men who see the figure have less wave length acuity than some that do not.

In plates 2, 3, 4 and 5 (see also Howell's *Textbook of Physiology*, 13 ed. p. 370) the dichromat sees the wrong figure because he can distinguish between blueness and red-greenness but not between greenness and redness and to the normal eye the red green contrast makes the best figure. The dichromat sees a bluish figure against a red-green background or vice versa. The trichromat with normal wave length discrimination sees the same figure as the dichromat but describes it quite differently. He sees a figure which is made up of dots that differ among themselves in color but are separated from the background by being more or less intense in color, more or less chromatic. He can tell each dot by its proper color name but uses, in his psyche, a different unifying principle in making a figure of them. Most of us speak, in looking at plate 3 for instance, of a reddish yellow six while he speaks of a five with a bluish top and a reddish bottom. He prefers to use unity of saturation rather than unity of hue in making up his patterns.

Plates 10 and 11 have a bluish figure on a very contrastly orange, red and green background. Most of us overlook the figure, since our eye follows the pattern in the background. The Ishihara blind trichromat with normal wave length

discrimination does not follow the brilliant background pattern but sees the figure. Some observers say the figure is bluish, some say that it is paleish and some that it is darkish.

The evidence, therefore, leads to the conclusion that the Ishihara and similar tests do not tell whether the individual tests can discriminate hue but give a rather confused index as to whether he is or is not anomalous as to the manner in which he arranges his visual field into patterns.

SUMMARY

Over half of those tested who are deficient in the Ishihara test have normal wave length discrimination.

Two dichromats did no worse with the Ishihara test than several subjects who had normal wave length discrimination.

The Ishihara test seems to evaluate a complex psychic bent rather than a sensory deficiency.

Dubious improvement occurred in the wave length discrimination of one individual out of thirteen under vitamin A administration.

Dubious improvement occurred in the Ishihara response of another individual under vitamin A administration.

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RELATION OF HEART RATE TO SLOW WAVES IN THE ELECTRO-ENCEPHALOGRAM DURING OVERVENTILATION

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In another study (1943) increase of slow waves in the electroencephalogram during overventilation was shown by us to be associated with an increase of heart rate. Inspection of polygraphic and electroencephalographic records generally revealed a correspondence in the time of appearance of the two changes. Better demonstration of the temporal relationship made simultaneous electrocardiographic and electroencephalographic recording on the same graph desirable. The present paper offers results of such simultaneous parallel recording of the two changes.

PROCEDURE. Human subjects were studied in the course of routine electroencephalographic examinations.¹ An Offner four channel resistance-capacity coupled amplifier and crystograph was employed. An additional magnetic marker and amplifier recorded the electrocardiogram, at low amplification, on the EEG recording paper. EKG leads consisted of metal clips wrapped in saline-soaked cotton placed in the arm pits. Overventilation was carried on for one minute periods, regulated by instructions from the operator. Changes in length of inter-R interval of the EKG are multiplied by 5 by using proportional dividers (Darrow, 1929; Lindsley and Sassaman, 1938) or a 5 to 1 wedge, and plotted downward from the baseline as a record of cardiac frequency. An electrical record from near the eyes assisted recognition of artifacts due to eye movement.

RESULTS. A typical record illustrating the slowing of the electroencephalogram and the simultaneous acceleration of the electrocardiogram is presented in figure 1. Additional EEG records along with graphs of simultaneous changes in cardiac frequency are presented in figure 2. These sample records illustrate 1, that the time at which slow waves markedly increase in the EEG can generally be identified; 2, that the appearance of slow waves tends to be simultaneous with, or preceded a few seconds by a definite increase in heart rate, and 3, that the sinus arrhythmia occasioned by the effort of overbreathing tends to disappear at the time of marked EEG slowing and EKG acceleration.

To demonstrate the consistency of this temporal relationship, data from a group of subjects having pronounced slow waves precipitated in the EEG during one minute of overventilation are presented in table 1. Cases with only moderately increased delta activity are, for present purposes, not considered. For this analysis the moment of definitely increased slowing of the EEG record was determined and cardiac frequency was measured for successive six second intervals before and after this change. The number of inter-R intervals for each six

¹ Subjects reclined in a darkened, shielded room.

second period was counted to the nearest 1/10th, multiplied by 10, and tabulated as heart rate per minute. Heart rate for successive six second intervals before and after beginning EEG slowing is presented. Change in rate between suc-

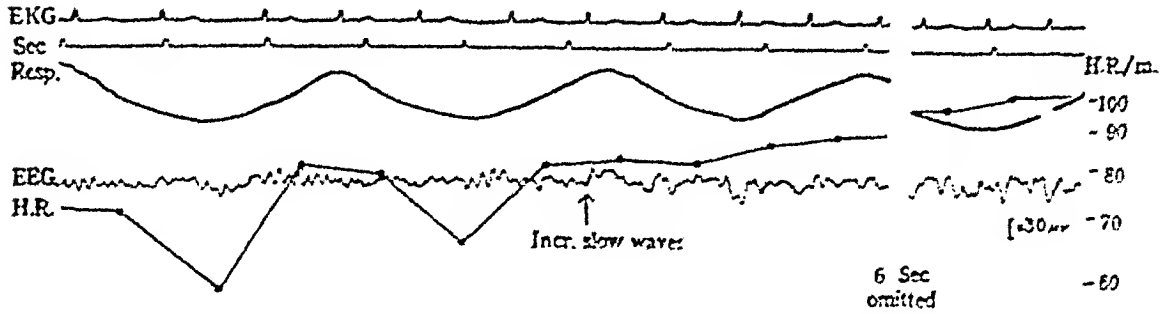


Fig. 1. Relation of heart rate to slowing of EEG. *EKG*, electrocardiogram *Sec*, seconds; *Resp*, respiration; *EEG*, Electroencephalogram of R. occipital area; *H.R.* heart rate plotted from EKG as described.

Figure shows 1, change in EEG at beginning of slow waves; 2, simultaneous increase of heart rate; 3, simultaneous decrease of sinus arrhythmia.

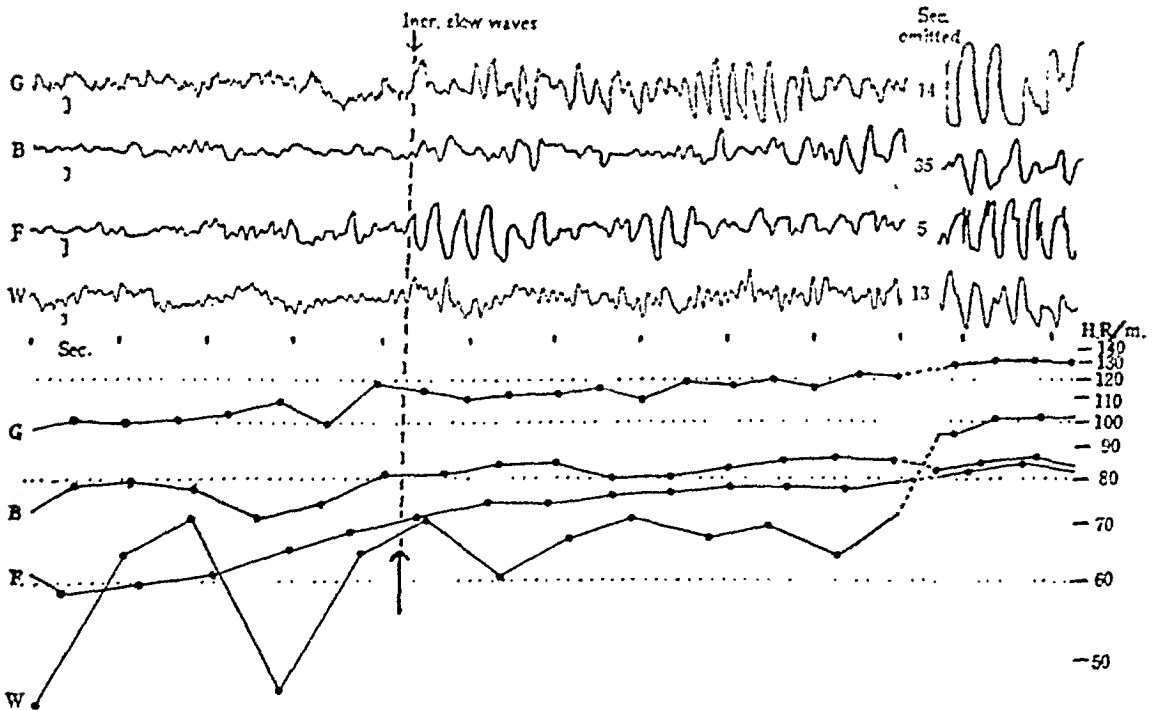


Fig. 2. Relation of heart rate to slowing of EEG; four subjects, G, B, F and W. Beginning slow waves at arrows. Simultaneous heart rates plotted as described. EEG calibration 30 microvolts.

cessive intervals, and total change in rate are shown in bold faced type. For each subject the maximum change between successive intervals is italicized. It will be noted that maximum cardiac acceleration rather consistently occurs with, or immediately precedes, slowing of the electroencephalogram. It will be

TABLE 1
Acceleration of heart rate related to marked slowing of EEG during overventilation

SUBJ.	HEART RATE PER MINUTE														MAX. RATE MINUS RATE BEFORE VENT.	RATE AT END MINUS RATE BE- FORE VENT.			
	Before slowing EEG						After slowing EEG												
	18"-12"		12"-6"		Change	6"-0"		Change	0"-6"		Change	6"-12"		Change			12"-18"	Before end vent.	Max. rate
	Before vent.†	Change	Change	Change		Change	Change		Change	Change		Change	Change						
D. D.	69	82	5	87	-9	78	15	93	7	100	0	100			100	100	31		
J. B.	70	72	8	80	3	83	10	93	2	95	*				95	95	25		
A. B.	90	98	4	102	5	107	12	119	-4	115	3	118			122	122	32		
P. C.	70	73	0	73	4	77	7	84	3	87	3	90			112	116	42		
J. S.	84	*		87	-7	80	15	95	5	100	-5	95			96	100	16		
K. W.	72	78	5	83	1	84	11	95	0	95	*				95	95	23		
S. M.	98	100	4	104	11	115	15	130	2	132	-8	124			122	132	24		
P. M.	80	90	0	90	0	90	17	107	7	114	4	118			105	118	38		
R. L.	71	74	-2	72	7	79	16	95	11	106	3	109			118	118	47		
H. T.	57	72	-6	66	4	70	16	86	3	89	6	95			95	95	38		
I. J.	89	97	-1	96	4	100	12	112	3	115	-2	113			105	115	16		
Ave. Accel.....																	32.4	28.6	

* Intervals coming before or after ventilation.

† Change of H.R. with beginning ventilation is here omitted as irrelevant.

Heart rate per minute is presented for successive six second intervals.

Change in rate between successive intervals is in bold faced type.

Maximum increase between successive intervals is in italicized bold faced type.

The table shows the cardiac acceleration which usually accompanies the appearance of pronounced slow waves in the EEG during over-ventilation.

TABLE 2
Heart rate in absence of slowing of EEG during overventilation

SUBL.	HEART RATE PER MINUTE AT SUCCESSIVE STAGES OF OVERVENTILATION										Before vent.*	Before end vent.	Max. rate	MAX. RATE MINUS RATE BEFORE VENT.	RATE AT END VENT. MINUS RATE BEFORE VENT.
	6°-12°	12°-18°	18°-24°	24°-30°	30°-36°	36°-42°	42°-48°	Change							
	Change	Change	Change	Change	Change	Change	Change	Change	Change						
W. S.	107	3	110	0	110	2	112	1	113	0	113	109	113	11	7
P. L.	87	1	88	2	90	0	90	-1	89	1	90	90	90	10	10
R. B.	104	-1	103	2	105	-5	100	3	103	-1	102	100	105	8	3
W. A.	96	1	97	2	99	3	102	1	103	0	103	93	103	10	0
L. D.	90	6	96	5	101	2	103	-1	102	-1	101	95	103	16	8
J. S.	59	2	61	5	66	1	67	4	71	1	72	72	78	19	13
E. B.	86	-2	84	6	92	1	93	1	94	1	95	92	95	9	6
W. N.	65	-4	61	1	62	0	62	-1	61	-2	59	61	62	0	-1
A. I.	73	2	75	8	83	-3	80	-2	78	-1	77	75	83	15	7
J. K.	74	6	80	-2	78	2	80	0	80	1	81	88	88	8	8
Ave. Accel.....		1.4	3.1	0.3	0.5	-0.1	-0.5							10.6	6.1

Average of italicized maxima 4.6.

* Change of H.R. with beginning ventilation is here omitted as irrelevant.

Heart rate per minute is presented for successive six second intervals.

Heart rate per minute between successive intervals is in bold faced type.

Maximum increase between successive intervals is in italicized bold faced type.

The table shows the relatively small increase of heart rate during overventilation in cases where there is absence of slow waves in the electroencephalogram.

noted that the total change in individuals with slowing of the EEG is consistently large.

This association of slowing of the EEG with acceleration of the EKG during overventilation is further emphasized by data in table 2 on cases in which *no slow waves* appeared in the EEG during the one minute of overbreathing. In the absence of a point of definite EEG slowing, table 2 presents a larger number of successive six second intervals than table 1, and the maximum heart rate attained during the entire period of overbreathing is also given. Change in frequency of successive intervals is bold faced as in table 1. The absence in most cases of any single point of pronounced cardiac acceleration, and the relatively small change in heart rate from beginning to end of ventilation is to be noted.

DISCUSSION. The evaluation of these results requires emphasis on the fact that these data are obtained *during overventilation*. Extended study of simultaneously recorded EEG and heart rate in the absence of hypocapnia reveals no such consistent inverse relation of heart rate to spontaneously appearing slow waves in the EEG. Even the reversal of effects during recovery from hypocapnia does not show the consistent temporal relationship observed for precipitation of the effects. Although at the end of overventilation, recovery of initial EEG and heart rate frequently occur together, there are also numerous instances in which slow waves in the EEG persist for an appreciable period after return of heart rate to normal. Rising blood pressure during recovery may be a significant factor in this discrepancy, tending, as it does, to slow the pulse while at the same time aggravating cerebral hypocapnia by increasing blood flow. Spontaneous bursts of slow waves also tend to occur after termination of overventilation without associated cardiac acceleration. It must also be borne in mind that during overbreathing cardiac acceleration frequently precedes slow waves in the EEG by two or three seconds. Seldom if ever does EEG slowing precede cardiac acceleration. Two subjects (E. B. and A. I.) in table 2 showed a point of appreciable acceleration without associated slow waves in the EEG. Furthermore, subjects in whom cardiac acceleration during overventilation is only transitory do not typically show the large increase of slow wave activity which is the basis for inclusion in this study. These observations suggest that we are not dealing with an immediate and direct effect of cardiac acceleration in the brain. They suggest, rather, that something has occurred with development of hypocapnia and in association with fall in blood pressure and cardiac acceleration to favor, but not necessarily to initiate, the development of slow waves.

In a previously mentioned paper (1943) it was shown that marked cardiac acceleration tends to occur during overventilation only in the presence of a fall in blood pressure, but that without cardiac acceleration, fall in blood pressure due to overventilation does not produce slow waves. The cardiac acceleration is apparently in compensation for the decreased blood pressure and presumably involves a decrease of vagal impulses. The associated disappearance of the sinus arrhythmia of respiratory effort (fig. 1 and fig. 2, subject W) supports this interpretation (Bond, 1942). A temporal relation of cardiac acceleration to the appearance of slow waves in the EEG suggests that we are dealing with a condi-

tion in which a diminution of vagal effects upon the brain may render the brain or its blood vessels more susceptible than otherwise to the constrictor action of hypocapnia.

That vagal stimulation may produce electroencephalographic effects in pre-frontal areas of the brain was demonstrated by Bailey and Bremer (1938). That it may produce dilatation of pial and ependymal blood vessels by way of connections through the facial nerve, geniculate ganglion and internal carotid nerve has been demonstrated by Cobb and Finesinger (1932); Chorobski and Penfield (1932); Putman and Ask-Upmark (1934); and Forbes, Nason and Wortman (1937).² That blocking of vagal impulses to the cerebral blood vessels may reduce cholinergic vasodilator influences at a time when destruction of acetylcholine by cholinesterase is accelerated by increased alkalinity (Gesell and collaborators, 1942, 1943) may be crucial. It is possible that during hypocapnia when destruction of acetylcholine is increased, any appreciable diminution of vagal cholinergic vasodilator impulses to the brain might precipitate spasmodic effects in cerebral vascular mechanisms. This would account for the observed relationship of EEG to heart rate during overventilation, the absence of a one to one temporal correspondence of the two, and explain the lack of any relationship when hypocapnia is absent.

SUMMARY AND CONCLUSION

It is demonstrated that in one minute periods of overventilation increase of heart rate tends to be antecedent to or simultaneous with slowing of the electroencephalogram. The relation of cardiac and cerebral effects during hypocapnia suggests a possible vagal mechanism. It suggests that reduction of vagal cholinergic vasodilator impulses at a time when destruction of acetylcholine by cholinesterase is increased by hypocapnia may be critical. Consequent contractions of the cerebral blood vessels may account for association of cardiac acceleration during overventilation with slow waves in the electroencephalogram.

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² As this goes to press we can report that E. W. Davis, H. W. Garol, J. R. Green, and C. W. Darrow, working in Doctor McCulloch's laboratory, have demonstrated the influence of this mechanism on the EEG. It is shown that in cats high potential slow waves following overventilation are greatly exaggerated after section of the facial nerve proximal to the geniculate ganglion.

THE EFFECT OF CRYSTALLOIDAL AND PROTEIN-CONTAINING SOLUTIONS ON THE BODY FLUIDS AND CIRCULATING PLASMA PROTEINS

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The purpose of the present study is to determine the effects upon the body fluids and total circulating proteins produced by the intravenous injection of commonly used crystalloidal and protein-containing solutions. Some of the phases of this problem have been investigated, but in some of these instances the more accurate methods of determining changes in the body fluids were not at that time available. Also, relatively little study has been directed toward the extracellular and intracellular fluids as affected by intravenous injections.

Normal saline and 5 per cent glucose were shown to produce a temporary increase in plasma volume by Blalock, Beard and Thuss (1). The increase was only a small portion of the total volume injected. Similar results were recorded by J. D. Robertson (2), Gilligan, Altschule and Volk (3) and Freeman and Wallace (4). Murphy, Correll and Grill (5) demonstrated a temporary marked increase in plasma volume after intravenous injections of hypertonic glucose in hospital patients. Freeman and Wallace (4) showed that normal plasma and hypertonic plasma in dogs led to an increase in plasma volume that was sustained for several hours. We (6) have previously shown also that hypertonic plasma in dogs leads to an increase in plasma volume which is proportional to the amount of protein injected.

Evidence relating to the effect of injection of fluids intravenously upon the plasma proteins is not entirely concordant. Miller and Poindexter (7) obtained a decrease of 20 to 52 per cent of total proteins. Blalock, Beard and Thuss (1) were unable to demonstrate any decrease in the total circulating protein in normal animals receiving crystalloidal solutions. Shearburn (8) reported an increase in circulating protein after large infusions. Several investigators (4, 6) have been able to demonstrate, on the other hand, an increase in total circulating protein after the injection of protein-containing solutions.

Coller, Dick and Maddock (9) have shown that solutions containing saline are retained in the body much more readily than solutions of glucose in water. Large increases in extracellular fluid have been produced in hospital patients by injecting glucose and saline solutions as reported by Stewart and Rourke (10). These investigators illustrated the tendency for glucose solutions to lead to ultimate dehydration. We have previously demonstrated an increase in extracellular fluid at the expense of cellular fluid, produced by the injection of hypertonic plasma.

Murphy, Correll and Grill (5) found a definite diuresis following the in-

travenous injection of glucose and saline solutions. This was most marked after 5 per cent glucose. Hypertonicity of the glucose solutions appeared to increase the diuresis.

In a recent study, the effect of normal saline, normal plasma, and hypertonic plasma was investigated in animals shocked by freezing a hind leg by Muirhead and associates (11).

METHODS. Normal fasting dogs which were anesthetized with pentobarbital sodium were used in this study. Control studies were made before the intravenous injections were begun and these consisted of plasma volume, available fluid for solution of thiocyanate which was taken to represent roughly the extracellular fluid, urine output by suprapubic catheter, and total circulating plasma protein. These studies were repeated as soon as possible after the intravenous injection was completed and also about 2½ hours after the completion of the injection. The plasma volume was determined by the Evans blue dye method (12); correction for disappearance of the dye was made (13). Extracellular fluid volume was taken to be represented approximately by the amount of fluid available for solution of thiocyanate (14). The determination of available fluid after the intravenous injection was made by reinjection of thiocyanate. The rate of disappearance of the thiocyanate from the extracellular space was determined separately in each animal and correction accordingly made in the calculation (15). Record of urine output, weight changes, and amount of fluid injected was kept in each experiment, so that it was possible to calculate roughly the changes in intracellular fluid. Total circulating plasma proteins were calculated from the total protein in grams per 100 cc. of plasma and the plasma volume. The Greenberg method (16), adapted to the Lumetron photoelectric colorimeter, was employed for protein determination.

The fluids were administered intravenously as follows: normal saline and 5 per cent glucose were given in volumes of 1000 cc. to each animal in their respective groups, an average of 100 cc. per kilo of body weight. The solution was administered by continuous drip and the average time required for the administration was 79 minutes. Concentrated plasma and 50 per cent glucose were administered by syringe. One hundred cubic centimeters of 50 per cent glucose was given to each animal in this group, an average of about 10 cc. per kilo, and the average time required for administration was 6 minutes. The concentrated plasma used was citrated human plasma. It was administered slowly to avoid citrate effect; the average time required was 20 minutes. These animals received an average of 12.6 grams of protein in 66 cc. of solution. Normal plasma was administered by continuous drip and an average of 20.8 grams of protein in 365 cc. of solution was administered to each dog in this group. The average time required was 50 minutes.

Both plasma preparations were obtained by diluting dried human citrated plasma, prepared by the Adtevac process (17), with distilled water in the required amount.

All determinations were carried out upon heparinized venous blood obtained from exposed jugular veins.

RESULTS AND DISCUSSION. The results of the four types of fluid upon plasma volume, extracellular fluid as available fluid for solution of thiocyanate, calculated intracellular fluid, total circulating plasma protein, and urine volume output are shown in tables 1 to 5. The results as presented compare control values with values almost immediately after, and approximately $2\frac{1}{2}$ hours after completion of the injection.

1. *Normal saline.* In dogs receiving normal saline the plasma volume was found to be significantly increased immediately after the injection. At this time, an average of 19.9 per cent of the injected 1000 cc. could be accounted for in the plasma. This increase, however, was much less pronounced 3 hours

TABLE 1

Data on four dogs receiving 1000 cc. of normal saline, each

Percentage changes are expressed in terms of the volume injected

DOG NO. AND WT. (KGM.)	PLASMA VOLUME CON- TROL	PLASMA VOLUME AFTER 16-99 MIN.*	PLASMA VOLUME AFTER 181-260 MIN.	CONTROL EXTRA- CELL. FLUID	EXTRA- CELL. FLUID AFTER 181- 260 MIN.	CONTROL CELLULAR FLUID	CELLULAR FLUID AFTER 181-260 MIN.	CONTROL PROTEIN	PROTEIN AFTER 181-260 MIN.	URINE VOLUME 181-260 MIN.
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	gm.	gm.	cc.
No. 1 10.6	505	695 +18%	660 +14.3%	3290	4000 +67.5%	4620	4861 +22.8%	27.8	25.2 -2.6	93 8.9%
No. 2 10.4	510	620 +11%	445 -6.5%	2660	3640 +98%	5140	4915 -22.5%	31.1	29.8 -1.3	245 24.5%
No. 3 10.6	520	690 +17%	500 -2%	3040	3240 +20%	4958	4808 -15%	30.6	30.4 -0.2	950 95%
No. 4 6.7	330	635 +34%	460 +14.5%	2110	2420 +34.4%	2940	3005 +7.2%	18.4	19.4 +1.0	500 55.5%
Average 9.5	466	658 +19.9%	516 +5.1%	2775	3325 +55%	4415	4398 -1.9%	27.0	26.2 -0.8	447 44.7%

* First figure represents time from completion of injection; second figure time from beginning.

later when an average of only 5.1 per cent of the injected fluid was still within the circulating blood. This finding is in accord with those of other investigators (1, 2, 4). These results would indicate that at the beginning of the injection, fluid commences to pass across the capillary membrane into the interstitial spaces, into the glomeruli, or into other extravascular locations. This rapid and marked passage of fluid might conceivably be expected to carry with it some of the circulating plasma protein since it has been demonstrated that the capillary membrane is not absolutely impervious to protein molecules (18). However, our results do not indicate any such significant disposition of the plasma proteins. The average change was a decrease of 0.8 gram. Naturally, the plasma protein concentration would be expected to and did change in accordance with the degree of hemodilution. From these findings we can say that there is

no significant loss of circulating plasma protein following the injection of normal saline in the animal with normal, intact circulation, even though large quantities of fluid pass across the capillary membrane.

The determination of available fluid requires longer than the plasma volume determination, hence at the stage immediately after completion of the injection it is not possible to say what the effect upon extracellular volume is. However, 3 hours after completion of the injection, an average of 55 per cent of the injected volume was detected in the extracellular space. At the same time, the increased urine volume had accounted for an average of 44.7 per cent of the injected fluid. Except for weight changes that could be accounted for by the amount of fluid injected, blood withdrawn, and urine output, there were no significant changes in the body weight. This was also true in the other experiments. Therefore,

TABLE 2

Dogs receiving 1000 cc. of 5 per cent glucose in distilled water, each

DOG NO. AND WT. (KGM.)	PLASMA VOLUME CON- TROL	PLASMA VOLUME AFTER 15-92 MIN.	PLASMA VOLUME AFTER 151-182 MIN.	CONTROL EXTRA- CELL. FLUID	EXTRA- CELL. FLUID AFTER 151- 182 MIN.	CONTROL CELLULAR FLUID	CELLULAR FLUID AFTER 151-182 MIN.	CONTROL PROTEIN	PROTEIN AFTER 151-182 MIN.	URINE VOLUME 151-182 MIN.
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	gm.	gm.	cc.
No. 5 8.1	317	390 +7.3%	300 -1.7%	2720	2940 +22%	3330	3685 +35%	21.1	18.0 -3.1%	425 42.5%
No. 6 11.5	575	620 +4.5%	600 +2.5%	3020	3370 +35%	5560	5575 +1.5%	34.5	30.0 -4.5%	635 63.5%
No. 7 9.4	407	428 +2.1%	408 +0.1%	2440	2020 -42%	4610	5330 +72%	19.2	22.8 +3.6%	700 70%
No. 8 13.7	550	550 0	508 -4.2%	3000	3260 +26%	7260	7460 +20%	36.8	38.6 +1.8%	540 54%
Average 10.4	470	494 +2.4%	454 -1.6%	2795	2895 +10%	5190	5512 +33.2%	27.9	27.3 -0.6%	575 57.5%

in animals receiving normal saline there was no significant change in the amount of cellular fluid detected by our methods of investigation. This aspect of the distribution of injected fluids having been previously uninvestigated, we can only say that it is in accord with expectations on the basis of principles of osmosis and semi-permeable membranes. Sodium and chloride ions being relatively unable to enter the cells, one would not expect injected normal saline to enter the cells.

2. *Five per cent glucose.* It will be seen in table 2 that 5 per cent glucose effects a less marked immediate rise in plasma volume than normal saline. Shortly after completion of the injection, an average of only 2.4 per cent of the injected fluid can be accounted for in the plasma, and at 2½ hours after completing the injection the plasma volume is back to normal if not slightly decreased. This obviously indicates an even more rapid escape of the injected fluid from the

plasma than with normal saline. Nevertheless, studies of the total circulating plasma protein fail to reveal any evidence of escape of plasma protein from the blood vessels. The increase produced in the extracellular fluid was relatively small and at $2\frac{1}{2}$ hours an average of only 10 per cent of the injected fluid was accounted for in the fluid available for solution of thiocyanate. On the other hand the urine volume at this same time accounted for 57.5 per cent of the injected fluid. This still leaves about 33 per cent of the injected fluid unaccounted for, and it is our assumption that this fluid entered the cells of the body. This might even be expected to occur since the cell membrane is permeable to the glucose molecule.

TABLE 3

Dogs receiving 50 per cent glucose solutions

Each animal received 100 cc. Changes in values are presented in terms of cubic centimeters

DOG NO. AND WT. (KGM.)	PLASMA VOLUME CON- TROL	PLASMA VOLUME AFTER 29-35 MIN.	PLASMA VOLUME AFTER 198-207 MIN.	CONTROL EXTRA- CELL. FLUID	EXTRA- CELL. FLUID AFTER 169- 178 MIN.	CONTROL CELLULAR FLUID	CELLULAR FLUID AFTER 169-178 MIN.	CONTROL PROTEIN	PROTEIN AFTER 198-207 MIN.	URINE VOLUME 198-207 MIN.
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	gm.	gm.	cc.
No. 9 8.1	335	595 +260	334 -1	2240	2180 -60	3810	3670 -140	20.2	20.2 +2.1	300
No. 10 13.8	675	615 -60	620 -55	3460	4880 +1420	6940	5295 -1685	36.8	36.2 -0.6	325
No. 11 12.7	735	695 -40	560 -175	3180	3400 +320	6320	5675 -645	39.4	37.2 -2.2	525
No. 12 7.2	280	263 -17	208 -72	1530	1240 -290	3870	3810 -60	16.2	14.0 -2.2	400
Average 10.4	506	542 +36	431 -75	2603	2925 +322	5235	4610 -625	28.4	27.4 -1.0	388

3. *Hypertonic glucose, 50 per cent.* The injection of hypertonic glucose in dogs can be shown by hematocrit study to result in a very rapid, short lasting plasma volume increase. However, since plasma volume determinations by the dye method require a longer time, it is not usually possible to demonstrate this increase completely. In one of our experiments we did obtain a fairly striking plasma volume increase but in others it was relatively slight, the average being an increase of 36 cc. Three hours later the average plasma volume was less than the control, a finding which has also been observed by others. The total circulating plasma proteins did not appear to be affected by the sudden plasma volume increment and rapidly occurring escape of fluid to the interstitial compartment. The average protein change was a decrease of 1.0 gram. In two animals, an increase in extracellular fluid occurred, in one a decrease, and in one no significant change. The average change was an increase of 322 cc., three times the amount

injected. The average urine output was 388 cc. Although these findings are not statistically significant, they correspond to the changes which would be expected from introducing a hypertonic solution into the extracellular compartment. Before time for equilibrium would be reached between the osmotic pressure in the cellular and extracellular fluids, there would be a period in which the hypertonic extracellular fluid would tend to draw fluid from the cells. It is readily seen that hypertonic glucose has an important dehydrating effect in that almost four times as much fluid is lost in the urine within a three hour period as was injected. At least a part of this fluid lost from the body seems to come from the cells.

4. *Plasma solutions.* In table 4 the results on dogs receiving injections of approximately normal plasma are shown. In these animals the plasma volume

TABLE 4
Dogs receiving approximately normal plasma

DOG NO. AND WT. (KGM.)	PLASMA GIVEN	PLASMA VOLUME CON- TROL	PLASMA VOLUME AFTER 24-78 MIN.	PLASMA VOLUME AFTER 180-225 MIN.	CON- TROL EXTRA- CELL. FLUID	EXTRA- CELL. FLUID AFTER 180-225 MIN.	CON- TROL CELL. FLUID	CELL. FLUID AFTER 180-225 MIN.	CON- TROL PROTEIN	PROTEIN AFTER 180-225 MIN.	URINE VOLUME AFTER 180-225 MIN.
No. 13 7.5	500 cc. 30.4 gm.	370	575 +205	620 +250	1750	2120 +370	3570	3950 +380	24.2	40.5 +16.3	12
No. 14 9.2	270 cc. 15.3 gm.	461	660 +196	596 +132	2730	3250 +520	4200	3700 -500	27.9	38.0 +10.1	200
No. 15 8.8	325 cc. 16.8 gm.	450	582 +132	585 +135	2125	2430 +305	4485	4328 -157	28.8	36.7 +7.9	177
Average 8.5	365 cc. 20.8 gm.	428	606 +178	600 +172	2202	2600 +398	4185	3993 -192	27.0	38.4 +11.4	130

was elevated immediately after the injection and this effect was well sustained during the succeeding three hours. An average of 20.8 grams of protein in 365 cc. was given to these dogs with an average increase of 178 cc. or 49 per cent of the injected volume. Three hours later there was no significant average change. At the same time the increase in total circulating protein averaged 11.4 grams, or 55 per cent of the injected amount. These results indicate that there is some immediate disposition of the injected protein and of a corresponding amount of water. Whether this disposition consists of a deviation to the interstitial space, the lymphatics, or to the so-called protein reserves of the body has not been determined. The extracellular fluid was found to be increased by the average amount of 398 cc., which closely corresponds to the volume of fluid added to the the extracellular space. However, there was an average loss of 192 cc. from the cells since there was a moderate increase of urine output. These results are in fairly good accord with what would be expected from injecting an isotonic solu-

tion into the extracellular space which contains protein molecules, except that the loss of some cell fluid is not readily explained.

With concentrated plasma, the changes produced in the body fluids were fairly similar to those previously reported (6). In table 5 it will be seen that there was a quick increase in the plasma volume amounting to an average of 134 cc. for 12.6 grams of protein in 65.8 cc. injected. With a basis of 6 grams of protein per 100 cc. of plasma this degree of dilution amounts to 63.5 per cent of the calculated blood diluting power of the injected proteins. This compares favorably with the blood volume increasing effect of whole or normal plasma. The increase in blood volume persisted in these dogs during the remaining 2½ hours of the experiment. These findings and those previously reported (6) as well as those of Freeman and Wallace (4), definitely indicate that hypertonic

TABLE 5
Data on dogs receiving hypertonic plasma

DOG NO. AND WT. (KGM.)	PLASMA GIVEN	PLASMA VOLUME CON- TROL	PLASMA VOLUME AFTER 32-52 MIN.	PLASMA VOLUME AFTER 143-167 MIN.	CON- TROL EXTRA- CELL. FLUID	EXTRA- CELL. FLUID AFTER 143-167 MIN.	CON- TROL CELL. FLUID	CELL. FLUID AFTER 143-167 MIN.	CON- TROL PROTEIN	PROTEIN AFTER 143-167 MIN.	URINE VOLUME AFTER 143-167 MIN.
		cc.	cc.	cc.	cc.	cc.	cc.	cc.	gm.	gm.	cc.
No. 16 7.0	80 cc. 15.5 gm.	328	500 +172	500 +172	1640	1720 +80	3610	3610 0	17.5	30.5 +13.0	10
No. 17 11.3	47 cc. 8.1 gm.	382	482 +100	505 +123	2320	2420 +100	6180	6057 -123	21.7	28.8 +7.1	70
No. 18 10.6	70 cc. 14.3 gm.	490	620 +130	620 +130	2400	2620 +220	5530	5293 -237	32.0	42.0 +10.0	87
Average 9.6	66 cc. 12.6 gm.	400	534 +134	542 +142	2120	2253 +133	5107	4987 -120	23.7	33.8 +10.1	56

plasma will produce an increase in plasma volume depending upon the amount of protein injected. The total circulating protein was increased by an average of 10.1 grams which represented about 80 per cent of the injected protein. The increase in extracellular fluid was found to average 133 cc., about twice the average volume injected. There was a decrease in cellular fluid of approximately the same volume possibly indicating that the hypertonic effect of the fluid injected into the extracellular space draws fluid from the cells. There was an average urine output of 56 cc. during the course of the experiment. This constituted a slight but definite diuresis.

CONCLUSIONS AND SUMMARY

It can be stated that injected normal saline effects a temporary rise in plasma volume, (most of) which has decreased after 3 hours; that this marked passage of fluid does not carry any appreciable amount of protein with it; that almost

all of the injected fluid is to be accounted for, at the end of three hours, in the extracellular space (by far predominantly the interstitial spaces) and in the increased urine output; and that there is no significant alteration in the cellular fluids following the injection.

With 5 per cent glucose, there is a less striking increase in the plasma volume, which has completely disappeared by the end of $2\frac{1}{2}$ hours. This rapid loss of fluid also fails to cause any loss of protein from the circulation. The extracellular space accommodates a small amount of the injected fluid at the end of $2\frac{1}{2}$ hours, but a considerable quantity has also entered the cells, presumably because the cell membrane is permeable to the glucose molecule. Five per cent glucose produces a quick and marked diuresis which accounts for over half of the injected fluid at the end of $2\frac{1}{2}$ hours.

Although hypertonic glucose produces a quick and very temporary increase in plasma volume, the methods employed for determination of plasma volume do not enable us to demonstrate this change in most cases. Twenty-nine minutes after the completion of the injection, the increase was very slight and by three hours the plasma volume was less than normal. It is suggested that the extracellular compartment is increased by withdrawing fluid from the cells. These body fluid shifts do not appreciably affect the circulating plasma proteins. Due to the diuresis produced by the small quantity of fluid, 50 per cent glucose is actually dehydrating to cells and to interstitial fluids.

Normal plasma and hypertonic plasma were both found to have a quick and sustained effect in increasing the plasma volume. The increase was proportionately the same in each instance depending upon the amount of protein injected. In neither case was the volume increase 100 per cent of the theoretical, and similarly there was some disposition of a part of the injected protein, the location of which is unknown. While normal plasma characteristically produced an increase in the extracellular fluid as a result of fluid volume injected, the same effect was obtained by hypertonic plasma, presumably by withdrawing fluid from the interstitial fluids and from the cells.

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RADIOACTIVE PHOSPHATE AS AN INDICATOR OF THE RELATIONSHIP BETWEEN THE PHOSPHATE CHANGES OF BLOOD, MUSCLE AND LIVER, FOLLOWING THE ADMINISTRATION OF INSULIN

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Several different concepts have been advanced to explain the phosphate changes in blood and tissues that follow the administration of insulin or epinephrine. Cori (1) believes that the inorganic phosphate which leaves the blood under the above conditions enters the muscle together with glucose to form hexose monophosphate. It has been known for a long time that insulin and epinephrine produce a decrease in the concentration of the inorganic serum phosphate (1-3) and an increase in the hexose monophosphate content of skeletal muscle (1, 4).

It was found that insulin has no effect on muscle hexose monophosphate in the adrenalectomized animal (4); however, the characteristic increase in muscle hexose monophosphate was observed following the injection of epinephrine. Therefore it was concluded by the Coris that when insulin acts on blood and muscle phosphates, it does so by inducing a reflex secretion of epinephrine consequent to the hypoglycemia caused by the insulin.

Soskin, Levine and Hechter (3), on the contrary, are of the opinion that the phosphate changes in blood and muscle are not directly related to each other. They believe that the fall in serum phosphate is due to insulin whereas the rise in hexose monophosphate content of muscle is due to epinephrine and results from a breakdown of muscle glycogen. These investigators suggest that insulin influences the esterification of inorganic phosphate elsewhere than in muscle. This idea is based on the slight rise they observed in the total acid soluble phosphate of whole blood following injection of epinephrine, and the fact that although insulin does not induce changes in muscle hexose monophosphates in the adrenalectomized animal, it still causes the characteristic reduction in the serum inorganic phosphate (3, 5).

In contrast to both the Cori and Soskin hypotheses, Nelson et al. (6) suggest that the decreased serum inorganic phosphate following the introduction of insulin can be largely accounted for by a rise in liver acid soluble phosphate.

In the work to be reported below, certain relationships were demonstrated between the phosphate changes in blood, muscle and liver following the administration of insulin, when radioactive inorganic phosphate was used as a tracer. Without the tracer technique, these relationships are difficult to discern.

EXPERIMENTAL METHODS. The experiments were carried out on normal and insulinized rabbits. The animals were placed under nembutal anesthesia. They were then injected with radio-active H_2HPO_4 containing approximately 1 mgm. of P by ear vein. The dosage of insulin was 0.75 unit per kgm. of body

weight. It also was introduced by the venous route and was administered immediately following the injection of the radioactive phosphate. The animals were sacrificed approximately 90 minutes after the injection of the labeled phosphate.

The inorganic, total acid soluble and barium soluble phosphate contents of blood, muscle and liver have been determined in the present work. Inorganic and total acid soluble phosphate were determined by the method of Fiske and Subbarow (7), the color being read on a photoelectric colorimeter. Muscle inorganic phosphate was determined after hydrolysis of the creatine phosphate according to the procedure of Cori and Cori (8). Hence the muscle inorganic phosphate values also include the phosphate from creatine phosphate. The barium soluble phosphate, which in muscle consists mainly of hexose monophosphates, was also determined by the method of Cori and Cori (8).

The inorganic P^{32} was determined by precipitating an aliquot of the barium insoluble fraction as magnesium ammonium phosphate using the magnesia mixture of Sacks mentioned by the Coris (9). Total acid soluble P^{32} was determined by evaporating an aliquot of the trichloroacetic acid extract to dryness in a Coors porcelain ashing capsule. The barium soluble P^{32} was determined in a similar manner. All radioactive samples were read on the Lauritsen electroscope.

Blood sugar was estimated by the method of Miller and Van Slyke (10).

RESULTS. Table 1 summarizes the changes in the phosphates of blood, muscles, and liver following the administration of insulin to rabbits.

Blood. The data show that there is considerably less inorganic P^{32} in the blood of the insulinized than in the control rabbits. This corroborates the observations that insulin has a marked effect in removing inorganic phosphate from serum.

The insulinized animals also exhibited an increase in the P^{31} and P^{32} values of the barium soluble fraction. This finding indicates that the rise which has been observed in the esterified blood phosphate (3) may be due to an increase in the blood hexose monophosphate fraction. The insulin apparently did not produce any change in the diphosphoglycerate content of the blood. This is of interest, since diphosphoglycerate constitutes a large part of the acid soluble phosphate of most mammalian red blood cells.

The decrease of specific activity of the total acid soluble phosphate in the blood of the insulinized animals indicates that insulin accelerates the transfer of phosphate from blood to other tissues.

Muscle. The total acid soluble P^{31} is not significantly changed following the injection of insulin but there is an increase in the total acid soluble P^{32} . This suggests that insulin may accelerate the rate of passage of phosphate both into and out of the muscle cell.

Our data confirm the previous findings that insulin causes an elevation in the hexose monophosphate content of muscle. The increased specific activity of the barium soluble fraction in the insulinized rabbits suggests that a large part of the P^{32} entering the muscle is transformed into hexose monophosphate.

Liver. Insulin produces a marked rise in the total acid soluble P^{31} and P^{32}

TABLE 1
Effect of insulin on the distribution of P_{21} and P_{22} in blood, muscle and liver of the rabbit*

NO.	TOTAL			INORGANIC			BARIUM SOLUBLE			BLOOD SUGAR
	pu	pu	S.A.	pu	pu	S.A.	pu	pu	S.A.	
Blood										
Control.....	51.3±0.8	122.4±4.6	24.0±0.51	5.0±0.16	64.0±2.2	128 ±7.5	4.3±0.37	11.8±0.57	28.0±2.1	411.5±5.7
Insulin treated.....	54.3±1.3	103.1±6.0	18.7±1.2	3.6±0.20	35.4±1.4	98.2±4.9	8.5±0.97	21.1±2.1	25.7±2.3	48.3±4.5
Muscle										
Control.....	139.7±2.5	68.1±0.92	4.9±0.40	92.1±3.4	52.2±1.4	5.7±0.33	14.0±0.90	7.4±0.28	5.2±0.25	717.87 730.41
Insulin treated.....	141.8±4.8	85.0±3.0	6.0±0.45	82.0±2.7	38.2±0.79	4.7±0.70	27.5±1.8	27.6±2.0	9.8±0.32	
Liver										
Control.....	79.0±2.5	215.9±14.7	27.2±1.2	19.1±1.14	71.1±6.1	37.4±0.95	37.4±0.81	61.7±6.2	17.3±1.4	
Insulin treated.....	94.6±2.0	297.1±14.2	31.4±0.88	24.6±1.21	87.4±3.1	35.5±0.51	42.4±2.5	90.1±4.6	21.9±1.2	

* P_{21} values are in milligrams per 100 grams fresh tissue. P_{22} values are in parts per thousand of the administered dose per 100 grams tissue. In blood, values are per 100 ml. whole blood. Measure of variability is standard error of the mean.

† S. A. = specific activity = $\frac{P_{22}}{P_{21}} \times 10$ of fraction.

of the liver. This agrees with the findings of Nelson et al. (6) whose investigations were carried out on the rat.

Further examination of the data shows that an increased specific activity of the barium soluble fraction of the liver results from the administration of insulin. In liver, the barium soluble fraction consists largely of substances other than hexose monophosphates. However, the data are of interest since they indicate that insulin produces an increase in phosphorylation processes in liver. In experiments with rats, we have found that insulin induces a marked increase in the radioactivity of the labile groups of the liver adenosine triphosphate (11).

DISCUSSION. The concept of the mechanism of insulin action derived from this study is in general consistent with the views of Soskin (12). However, the results indicate that insulin facilitates the transfer of serum inorganic phosphate into muscle and liver. This is in contradiction to the view of Soskin et al. (3) that muscle and serum phosphate changes following insulin administration are not related. The results agree with the findings of Nelson and co-workers that liver takes up a large amount of inorganic phosphate following the injection of insulin. The diminution observed in the serum inorganic phosphate probably is directly due to the insulin and not to the reflex secretion of epinephrine.

The Cori and the Soskin group attribute the rise in muscle hexose monophosphate following the injection of insulin to the reflex secretion of epinephrine. There is no reason to doubt such a view since even glucose administration does not produce any rise in muscle hexose monophosphate (1). There is also no increase in the hexose monophosphate content of the liver following the administration of glucose (1). Furthermore, Cori and Cori (9) have shown that epinephrine produces an accelerated breakdown of muscle glycogen into hexose monophosphates *in vitro*.

Insulin causes an increase in the hexose monophosphate content of the blood. This rise cannot be attributed to secondary epinephrine action. This is evident since blood contains only traces of glycogen. Therefore the increased hexose monophosphate level in blood must be brought about by the phosphorylation of glucose (by the adenylic acid system).

The action of insulin in increasing the phosphorylation of glucose is not limited to blood. Indeed, in order for glucose to be converted into glycogen, or to be utilized, it first must be phosphorylated. Soskin and co-workers (13) have demonstrated a stimulatory effect of insulin in the phosphorylation of glucose in skeletal muscle slices. The non-accumulation of hexose monophosphate in conditions such as adrenalectomy or after glucose administration does not necessarily mean a decreased ability to phosphorylate glucose under these conditions. In the intact animal blood glucose which is phosphorylated in liver and in muscle is either rapidly converted into glycogen or is immediately oxidized. Therefore the influence of insulin on the formation of hexose monophosphate from blood glucose cannot be detected in liver or muscle *in vivo*. It is possible that some accumulation of hexose phosphates may take place in the blood following insulin administration because of the absence of phosphorylase activity in blood.

SUMMARY

1. The changes in the phosphates of blood, muscle and liver produced by insulin have been studied by means of tracer experiments with radioactive phosphate.

2. Insulin causes an increase in the total acid soluble P^{32} of the liver and muscle; an increase in the total acid soluble P^{31} was noted only in the liver. The rate of disappearance of inorganic P^{32} from blood is accelerated by insulin.

3. Insulin induces a rise in the P^{31} and P^{32} of the barium soluble fraction of blood, muscle and liver. The specific activity of this fraction is increased in muscle and liver but not in blood. The rise in the barium soluble fraction in the blood is probably due to an increased esterification of glucose. In the muscle the increase mainly represents newly synthesized hexose monophosphate. The barium soluble fraction of the liver contains very little hexose monophosphate; the main component may be glycerol phosphate.

4. Evidence is presented which indicates that insulin produces similar effects in blood, muscle and liver.

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THE EFFECT OF THIAMINE DEFICIENCY AND OF REDUCED FOOD INTAKE ON RESISTANCE TO LOW OXYGEN TENSION IN THE CAT

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While engaged in a study of the physiological effects of thiamine deficiency upon cats, it was noted that this treatment appeared to increase markedly their resistance to low oxygen tension. This seemed to be correlated with the partially starved state or negative caloric balance resulting from the anorexia following the withdrawal of thiamine from the diet, a matter which seemed to warrant further investigation. After some preliminary experimentation we decided to approach the problem in the following manner. The length of time a cat could maintain respiration in an atmosphere of 3.25 per cent oxygen was taken as an index of its resistance to low oxygen tension. This time was determined by placing the animal in a small metal box, glazed on one side for purposes of observation and just large enough to accommodate the cat comfortably, but small enough to prevent it from moving about. Through this box air diluted by nitrogen to a point where its oxygen content was 3.25 per cent was forced under slight positive pressure from a large spirometer containing 108 liters of gas. This mixture passed through the box at a rate of about 4 liters per minute, which prevented the carbon dioxide content of the air in the box from rising above 0.3 per cent. In such an atmosphere and under such conditions a normal cat would maintain respiration from 15 to 60 minutes. The oxygen content of the air in the spirometer was usually determined for each experiment, to insure its being at the proper concentration.

Immediately after placing the cat in the chamber it was rapidly flushed out by the gas mixture described. This flush-out took about 5 minutes and at the end of this period the atmosphere in the chamber was the same as that in the spirometer. During this time the respiration was rapidly increasing from a basal rate of about 40 movements per minute to something between 100 and 200 movements per minute. The maximum respiratory rate typically was attained in 15 minutes and from here on it either remained relatively constant for some time or else began a slow decline. The final failure of the respiration was heralded by a sharp drop in the respiratory rate, which up until this time had been declining slowly and regularly. With a little experience the observer could easily recognize the signs of the approaching failure and predict within a minute or so when the final collapse would occur. Once complete respiratory failure was established, the cat was removed from the box and revived by artificial respiration. As a rule this never took longer than a minute and usually 15 to 30 seconds sufficed. The cat was on its feet within 10 to 15 minutes after removal, and within a few hours was none the worse for the experience.

One exposure a week was the rule for each cat, thus allowing ample time for recovery between tests as well as preventing any acclimatization. Except for 10C and 14D, as will be discussed later, none of the cats showed any tendency to increase their survival time during the control periods, indicating a lack of adaptation to the conditions within the chamber. All experiments were done in the post-absorptive state and the cats were weighed before each experiment. Feeding occurred regularly late in the afternoon after the experiments scheduled for that day had been performed.

The experiments were designed to show whether any correlation between caloric intake and resistance to low oxygen tension could be demonstrated. To this end the animals were given three different types of diet for varying lengths of time, usually two to three weeks, to determine the length of time respirations could be maintained in an atmosphere of 3.25 per cent oxygen on each diet. The three diets were 1, a control diet; 2, a thiamine deficient diet, and 3, a reduced diet.

The control diet consisted of enough canned rabbit meat to maintain a constant weight or to permit a return to the animal's usual weight after having been on either the thiamine deficient or the reduced diet. The thiamine deficient diet consisted of the same canned rabbit meat autoclaved at 15 pounds pressure for 5 hours. Previous studies¹ in this laboratory (Everett, 1913) have shown that such a diet produces thiamine deficiency in cats within 5 to 7 weeks. In the earlier experiments on the effects of low oxygen tension a few cats were carried to the point of convulsive seizures, but in the work reported here the diet was discontinued after the animal had been on it for 3 weeks or less. The thiamine deficiency acted to decrease the appetite of the animal to the point where the caloric intake was voluntarily reduced to a level resulting in weight loss. The anorexia of thiamine deficiency appears early, usually within the first week of the diet, and weight losses were recorded within the same length of time. On returning to normal diet these weight losses were fully or almost fully restored. The reduced diet was the same as the control diet, reduced sufficiently in amount to put the cats on a negative caloric balance. To do this necessitated giving about one-fourth of the amount of food usually fed supplemented with weekly doses of thiamine and other B vitamins.

RESULTS. The results of the experiments are summarized in figures 1 and 2 and table 1. The figures show graphically the individual history of each cat used. The length of time the animal maintained respiration during each weekly test is plotted on the graphs, together with its weight as determined immediately before the test. As shown, each cat was given a series of preliminary control tests over a period of two or three weeks to determine its resistance to low oxygen tension on a nutritionally adequate diet. Table 1 gives the mean survival times for each control and experimental series presented in figures 1 and 2 in the order in which the tests were conducted.

¹ We are indebted to Dr. D. F. Robertson and Mr. L. J. Ruland of the Merck Company for generous supplies of crystalline vitamins used in this work.

Following the control tests the animal was put on the thiamine deficient diet and in every instance an increase in resistance was noted in the first week. This diet was maintained two to four weeks depending on circumstances after which the cat was again returned to its usual food. During this second control period of two to three weeks the weight lost during the period of thiamine deficiency was regained either all or in part. Recovery being considered complete the cat was next placed on a reduced diet where again except for 13E, figure 2, marked increases in resistance to low oxygen tension quickly developed. In some cases,

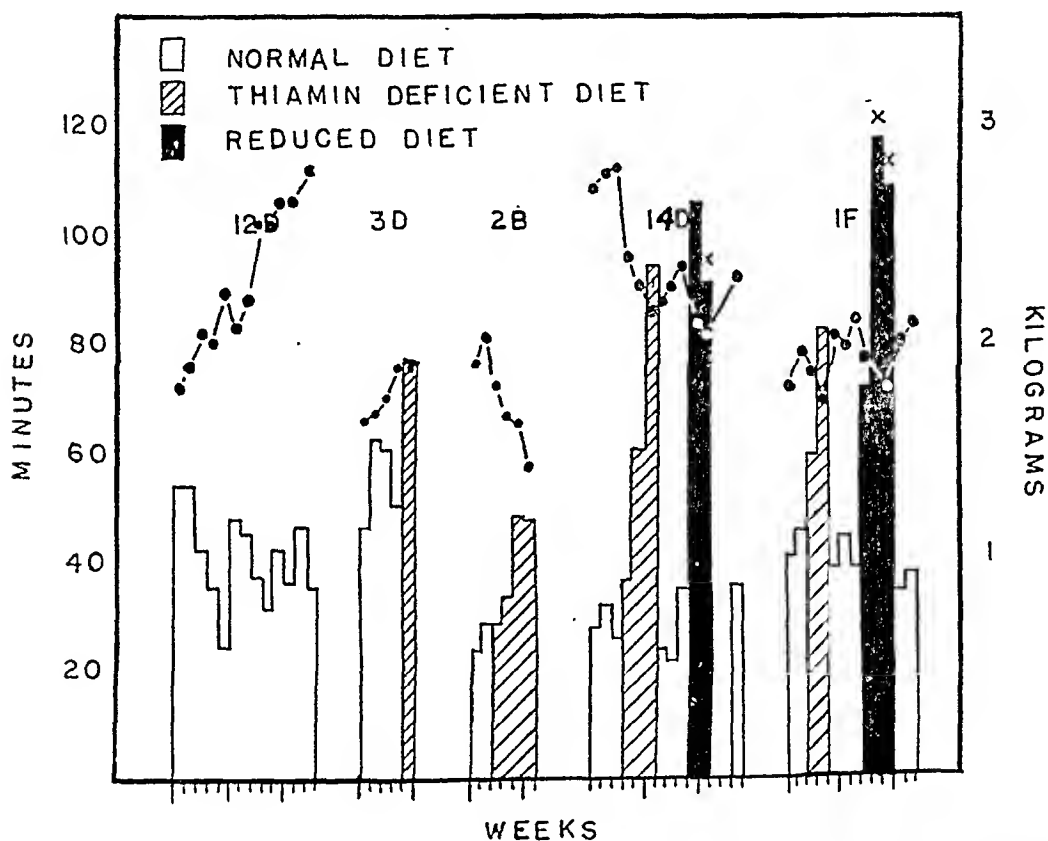


Fig. 1. Showing the length of time in minutes that respiration could be maintained in an atmosphere of 3.25 per cent oxygen together with weight changes in five cats kept on various experimental and control diets.

those marked with an X in the figures, the cats were removed from the respiration chamber before they had lost consciousness as they gave every sign of maintaining respiration indefinitely.

The fact that an increased resistance to low oxygen tension appeared in the animals on a reduced food intake make it doubtful whether on the thiamine deficient diet the effect was specifically due to a lack of thiamine. This view is further strengthened when it is recalled that severe signs of thiamine deficiency require 5 to 6 weeks to develop in the cat. The occasional gaps appearing in the weekly test records (14D, fig. 1; 10C, fig. 2) are due to illnesses occurring at the time when such tests would have been run. One control cat was run through-

out the period of the other experiments (12D, fig. 1) to check the possibility of some unforeseen environmental change influencing the results. Thirteen weekly tests gave no indication of any progressive increase or decrease in the resistance to low oxygen tension.

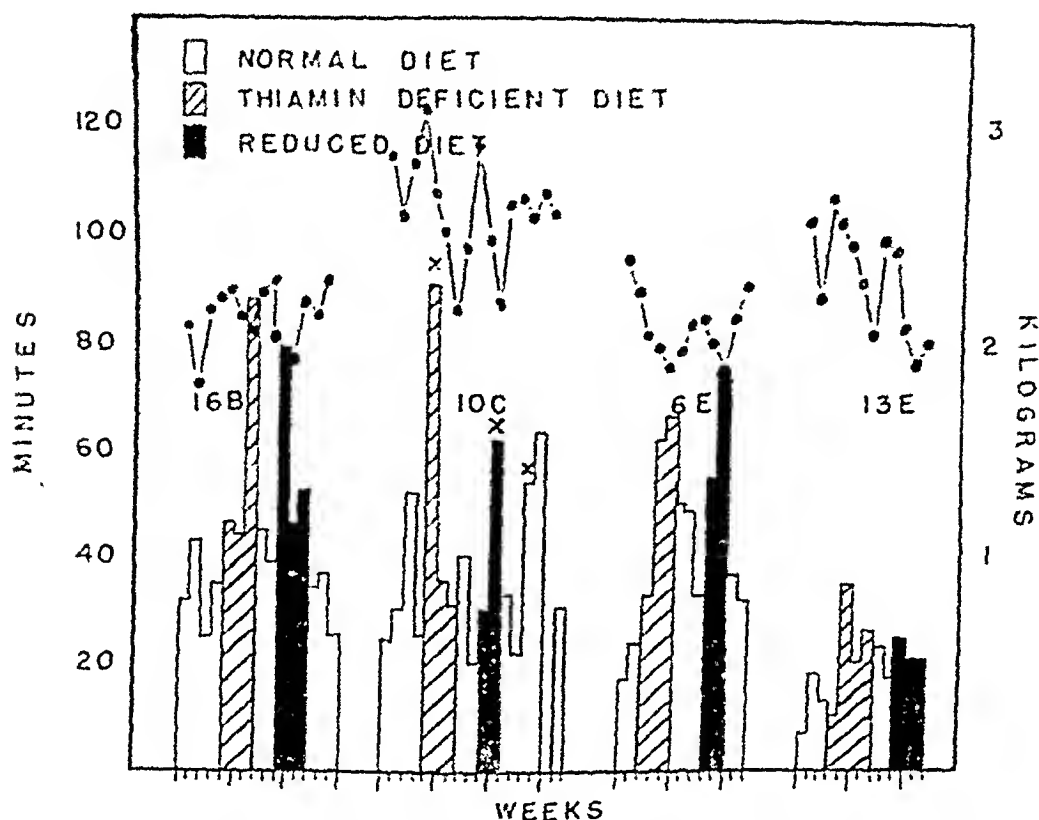


Fig. 2. Showing the length of time in minutes that respiration could be maintained in an atmosphere of 3.25 per cent oxygen together with weight changes in four cats kept on various experimental and control diets.

TABLE 1

Mean survival times of cats exposed to 3.25 per cent oxygen when kept on varying control and experimental diets

DIET	NUMBER OF TESTS	SURVIVAL TIME
		minutes
1, Control.....	29	33
Thiamine deficient.....	23	50
2, Control.....	14	36.8
Reduced diet.....	15	63.7
3, Control.....	13	36.6

The results were analysed statistically by means of Fisher's "t" test for small samples (Tippett, 1937), comparing the longest survival times for each animal during the experimental period with the longest survival during the immediately preceding control run. For the eight thiamine deficiency runs the value of "t"

was found to be 5.87 which is well above the value of 3.49 for the 0.01 level of significance and for the six reduced diet tests the value of "t" was 3.49 lying well above the 2.44 value for the 0.05 level of significance but slightly below the 3.70 value for the 0.01 level of significance. These results make it extremely unlikely that the observed effects could be due to chance alone.

DISCUSSION. Campbell (1938) showed that white rats after six days on an exclusive diet of carrots increased their resistance to low oxygen tension. In these experiments, the pressure of air in a decompression chamber was lowered to 240–250 mm. Hg to give oxygen tensions of about 50 mm. Hg, corresponding to about 5½ per cent oxygen at sea level. As an index of resistance, the number of survivors in the decompression chamber after 35 minutes was noted and while at the beginning of the diet this was only 12.5 per cent, at the end it was 100 per cent. He concludes that the weight loss on the carrot diet, which was 10 per cent to 20 per cent, was not an important factor in this increased resistance. Nelson et al. (1943) have also found increased tolerance to a simulated altitude of 30,000 feet in white rats on carrot diet. Their test animals suffered 11 per cent weight losses while controls were gaining weight. In a later paper Campbell (1939) extended his observation to other diets and from information so obtained concluded that the effect of the carrot diet was due to its low protein content, as well as to the presence of a large amount of fiber, glutamine and an unknown factor. As to the effects of proteins, he concluded that zein and gelatin diets increased resistance to low oxygen tension, while of the amino-acids tryptophane, tyrosine, lysine, glutamine gave protection, and histidin, arginine and cystine did not.

At the present time we are not prepared to speculate as to the cause of the increased resistance observed in our cats. Suffice it to say, this change appears to be related to some change in physiological state induced by the reduced caloric intake. What this change may be is a matter for further investigation. There are a number of variables in the blood chemistry capable of increasing resistance to anoxia which may also be related to a decreased food consumption. Campbell's dismissal of reduced food intake as being of no importance in the rat we feel is hardly justified under the circumstances. Complete starvation for one to two days does not seem to us a suitable control for a series of experiments running for six days on a carrot diet, even though the weight loss in each case might be comparable. According to Campbell his rats withstood starvation so poorly that they usually died at the end of the second day.

SUMMARY

Cats kept on a thiamine deficient diet for one to two weeks showed on the average an approximate increase of 50 per cent in the length of time they were able to maintain respiration in an atmosphere of 3.25 per cent oxygen.

Cats placed on a reduced diet such that they lost 15 to 20 per cent of their body weight within one to two weeks showed an even greater average increase in their resistance to low oxygen tension.

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THE RATE OF DECLINE IN RESISTANCE TO ANOXIA OF RABBITS, DOGS AND GUINEA PIGS FROM THE ONSET OF VIABILITY TO ADULT LIFE^{1, 2}

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The present investigation was undertaken to determine how long newborn rabbits, dogs and guinea pigs can survive when breathing nitrogen instead of air. Embryologists have long recognized the fact that when deprived of oxygen, the fetus survived longer than the mother under similar circumstances.

Certain previous investigations are of special interest. Le Gallois (1), in 1813, noted that in newborn rabbits, following decapitation, respiratory movements persisted for 15 minutes; in asphyxia by submersion, 27 minutes; after opening the thorax, 30 minutes; and following extirpation of the heart, 20 minutes. Thirty days after birth, the adult type of response was found and respiration ceased within 2 minutes.

Paul Bert (2) observed in newborn rats the persistence of respiratory movements for 30 minutes during submersion in water. Twenty days after birth, respiration ceased in about $1\frac{1}{2}$ minutes. He also noted that cat litter mates submerged in water at 20 degrees survived 26 minutes, while at 36 degrees the time of survival was only $11\frac{1}{2}$ minutes.

Observations have also been made upon animals placed in a bell jar into which various gases were introduced. By this method, Reiss and Haurowitz (3) and Avery and Johlin (4) showed that newborn mice were more resistant than adults, whether anoxia was produced by carbon monoxide, hydrogen, carbon dioxide or cyanide. The work of Fazekas, Alexander and Himwich (5) further substantiated this fact.

Kabat (6) found that, after interruption of the blood supply of the brain, the time of survival of the newborn dog was four times that of the adult. By ligation

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of vessels supplying the brain, as well as by decapitation, Selle and Witten (7) observed that the duration of gasping movements decreased with age.

The foregoing investigations have been limited to animals at term and during the neonatal period, and do not include observations on the survival time at earlier stages of development. The observations to be reported in this paper deal largely with the resistance to anoxia at various stages of fetal life and center about five main questions:

1. When nitrogen instead of air is breathed, what is the survival time of the normal newborn rabbit compared with that of the adult?
2. Is survival time increased or decreased in premature fetuses?
3. If the newly born survives longer than the adult, what is the duration of the period of transition from the newborn to the adult type of response?
4. Does a 3-day old rabbit living in the nest survive as long as a postmature fetus living in the uterus 3 days past term; or what is the influence of intrauterine environment on survival time?
5. In the comparison of various species (rabbit, guinea pig and dog) what is the relation of the stage of development and length of gestation period to survival time?

METHODS AND MATERIAL. Kymograph records of the respiratory movements were obtained on every animal throughout the experiment. In the smaller, newborn animals a plethysmograph connected with a recording tambour was used, as previously described by Rosenfeld and Snyder (8). The respiratory movements of the thorax and abdomen were readily observed through the transparent glass chamber which surrounded the animal. Rubber sheeting was fastened over the end of the above chamber and the head of the animal projected through a hole in the sheeting, of such size as to fit snugly around the neck without leakage of air and yet not so tight as to obstruct respiration. A second outer chamber of glass through which nitrogen circulated briskly was then placed over the end of the first chamber and the head of the animal. A similar arrangement using rubber sheeting as described above was used to prevent leakage of nitrogen. In adult animals, too large for the plethysmograph, respiration was recorded by fastening a small blood pressure cuff around the thorax.

In every animal a record of normal respiration was first obtained. Following this the chamber through which 99.9 per cent nitrogen was being circulated was slipped over the end of the plethysmograph and over the head of the animal. The time of survival was measured from the onset of nitrogen administration until the cessation of respiration as recorded on the kymograph tracing. Observations were made throughout at room temperature, about 70°F.

In fetuses at 29 to 35 days which were delivered by hysterotomy, observations on the litters were begun promptly following birth in order to detect any changes which may occur at this time.

Observations were based upon 174 rabbits obtained from 64 litters at various stages of development ranging from 29 days after conception to adults; 50 guinea pigs obtained from 22 litters; and 28 dogs from 6 litters.

The rabbits were reared in the laboratory and developmental stages were

known with accuracy since ovulation regularly occurs 10 hours after mating. Furthermore, since labor can be inhibited by injecting extract of pregnancy urine a week before term, it is possible to obtain fetuses of extreme postmature development, which have survived in the intrauterine environment 3 days past term (9).

OBSERVATIONS. 1. *Rabbit*. A. *Survival time of rabbits in nitrogen from prematurity to adult stage*. From the beginning it was evident that rabbits born at full term, 31 to 33 days, possessed a striking tolerance to anoxia. When nitrogen was breathed instead of air, at 31 days, respiratory movements continued 34 minutes as an average in 18 newborn animals obtained from 4 litters. At 32 days breathing continued 31 minutes in 14 animals obtained from 3 litters; and at 33 days, the time of survival averaged 27 minutes in 12 animals obtained from 4 litters. In sharp contrast to these, 6 adult rabbits survived only $1\frac{1}{2}$ minutes in nitrogen. Further observations showed that the adult type of response was reached 18 days after birth or about the end of the suckling period. At intermediate stages of development, the time of survival in nitrogen gradually diminished as the age increased. In table 1 are summarized the results from a series of 174 rabbits obtained from 64 litters.

Interesting light is thrown upon the oxygen requirement of fetuses long before birth by observations on fetuses delivered by hysterotomy during the different stages of pregnancy. Resistance to anoxia observed at the onset of viability at 29 days averaged about 30 times as great as in adult rabbits. Thus, in 16 fetuses obtained from 5 litters at 29 days, the time of survival was 44 minutes; in 9 fetuses obtained from 3 litters at 30 days, breathing continued for an average of 41 minutes.

The remarkable tolerance of premature rabbits to anoxia was further borne out by the fact that some were reared to adult life after more than 30 minutes in nitrogen. In these experiments, fetuses were removed from the nitrogen chamber before the final phase of increase in respiratory rate occurred, although a half hour or more had elapsed. Respiratory movements were resumed and, in some cases, a normal development to adult life was noted.

During the course of administration of nitrogen, samples of arterial blood were taken to determine the oxygen and carbon dioxide content, using the method of Van Slyke and Neill (10). Blood was obtained from the carotid arteries, or in certain instances by left ventricular puncture. After two minutes of nitrogen inhalation there was extreme anoxemia, the oxygen content being less than 3 volumes per cent. The carbon dioxide content determined at various intervals during the experiments in different animals was 30 to 60 volumes per cent.

B. *The time of survival of postmature rabbit fetuses*. By the experimental prolongation of pregnancy, fetuses were obtained which had survived in the intrauterine environment for 35 days, or 3 days past term. The question arises whether or not the survival time was altered by the prolonged period of three days within the uterus in contrast to fetuses which had lived in the nest for three days following birth at the normal 32 day term.

Observations revealed no increase in time of survival in nitrogen of animals

living in the intrauterine environment rather than outside of it. In rabbits which had lived in the nest for 3 days following birth at 32 days, the time of survival averaged 17 minutes in 13 animals obtained from 5 litters (table 1). In 6 animals of a litter obtained by hysterotomy at 35 days following experimental prolongation of pregnancy it was found that the time of survival averaged 16 minutes. In addition, in 7 animals obtained from 4 litters following spontaneous prolongation of gestation, the time of survival averaged 17 minutes (table 2). There was evidence that the tolerance to anoxia was related to the stage of devel-

TABLE 1
Survival time of rabbits of various ages

AGE	NO. OF LITTERS	NO. OF ANIMALS	AV. SURVIVAL TIME IN N ₂	AV. WT.
<i>days</i>			<i>min.</i>	<i>grams</i>
20	5	16	44	32
30	3	9	41	38
31	4	18	34	42
32 (term)	3	14	31	47
1	4	12	27	52
2	4	12	20	64
3	5	13	17	53
4	2	4	13	79
5	4	10	11	85
6	4	12	13	90
7	2	6	9	101
8	2	6	7	125
9	2	4	9	111
10	2	4	7	160
11	2	4	5	147
12	1	3	3	128
13	1	2	2.5	230
14	2	5	4	190
15	1	4	2.5	179
16	2	3	2.5	193
17	2	4	2.5	196
19	1	3	1.5	212
Adult	6	6	1.5	696

TABLE 2
Survival time of operatively and spontaneously delivered rabbits

AGE	NO. OF LITTERS	NO. OF ANIMALS	AV. SURVIVAL TIME IN N ₂
<i>Operative delivery</i>			
<i>days</i>			<i>min.</i>
20	5	16	44
30	2	6	41
31 (term)	4	18	31
33	2	7	27
34	2	6	22
35	1	6	16
<i>Spontaneous delivery</i>			
30	1	3	39
32 (term)	3	14	31
33	2	5	26
34	3	9	20
35	4	7	17

opment of the fetus rather than to the environment, whether within the uterus or outside of it.

Observations were also made at various stages of development of animals born normally in contrast to those obtained by hysterotomy. As shown in table 2 the type of delivery, whether operative or spontaneous, had no definite influence upon the time of survival in nitrogen at the various developmental stages studied.

2. *Guinea pig.* Observations on guinea pigs revealed striking differences in the findings with rabbits. The time of survival in nitrogen averaged 4½ minutes in 11 animals of 4 litters examined within 12 hours following spontaneous birth.

In fetuses obtained before labor by hysterotomy near term, the survival time averaged 6 minutes in 9 animals of 3 litters (table 3).

The period of transition to the adult type of response is also markedly shortened. At 7 days following birth the time of survival in nitrogen reached the adult level, namely, 3 minutes. It may be noted, however, that the survival time of the adult guinea pig was twice that of the adult rabbit.

3. *Dog.* The resistance of newborn dogs to anoxia was found to be of the same magnitude as that observed in rabbits at birth, although the duration of intrauterine life of the dog is twice that of the rabbit (table 4). A gradual decrease in survival time occurs until the adult type of response is reached during the fourth week following birth. Adult dogs survived in nitrogen for 3 minutes, or twice as long as adult rabbits.

TABLE 3

Survival time of guinea pigs at various stages of development

AGE	NO. OF LITTERS	NO. OF ANIMALS	AV. SURVIVAL TIME IN N ₂	AV. WT.
<i>days</i>			<i>min.</i>	<i>grams</i>
Term	3	9	6	66
0.5	4	11	4.5	88
1.0	4	6	4.5	83
1.5	2	3	4	109
2	1	3	4	94
3	1	3	4	99
4	3	4	3.5	100
5	2	4	3.5	123
6	3	4	3.5	155
7	3	3	3	170
8	3	3	3	185
Adult		5	3	952

TABLE 4

Survival time of dogs of various ages

AGE	NO. OF LITTERS	NO. OF ANIMALS	AV. SURVIVAL TIME IN N ₂	AV. WT.
<i>days</i>			<i>min.</i>	<i>grams</i>
0.5	2	3	31	223
1.5	2	5	25	210
4	2	4	17	347
7	2	3	14	285
14	3	5	8	614
21	1	2	5	900
28	1	3	3	1225
Adult		2	3	9000

DISCUSSION. The foregoing observations provide abundant evidence of a remarkable defense against asphyxia in the fetus. In all species examined it is evident that at birth there is a special capacity for maintenance of life in the mammalian organism under anaerobic conditions. This is especially striking in species such as the rabbit and dog, which are born in a primitive state of development. Evidence that resistance to extreme oxygen want decreases as structural organization becomes more highly differentiated was brought to light with clarity in rabbits in which observations were carried back to the earliest stages of viability. It was noted that the greatest tolerance to oxygen deprivation in the present series was shown by embryos at the stage when survival outside the intrauterine environment first became possible. Additional evidence in support of the finding that ability to resist anoxia is related to the stage of development rather than external environmental factors was obtained in postmature fetuses which had

been retained within the uterus until the extreme limit of intrauterine existence had been attained. Throughout the period of experimentally-prolonged intrauterine existence there was a steady loss of resistance to anoxia parallel to that which occurred in the external environment of animals at identical stages of development.

The present experiments demonstrate apparently for the first time the magnitude of the changes in resistance to anoxia which occur at various stages of intrauterine life from the onset of viability to extreme postmaturity.

In the neonatal period, the differences in the time of survival in nitrogen of the three species under consideration may be clarified by taking into account the differences in structural differentiation of the dog, rabbit and guinea pig at the time of birth. Although the gestation period of the dog is twice that of the rabbit, and the same as that of the guinea pig, the guinea pig is the only one of the three species which is sufficiently well developed at birth to carry on an independent existence. The newborn guinea pig shows advanced muscular co-ordination, running about the cage and devouring solid food; the eyelids are open and the development of the integument is marked by a conspicuous coat of long hair. In contrast, in the dog and rabbit at birth, muscular co-ordination is deficient, locomotion is limited, nutrition is dependent upon suckling, the eyelids are closed, and integumentary development is characterized by scant growth of hair. It is evident that a clue to the similarity in resistance to anoxia between the rabbit and dog (both species surviving one-half hour in nitrogen at birth in contrast to six minutes in the case of the guinea pig) is found in the close resemblance of these two species in respect to the stage of development at birth despite a marked difference in the length of intrauterine life.

The fact that fetuses possess a defense against anoxia—which in dogs and rabbits at birth amounts to ten times or more that of the adult—throws light upon the metabolic processes by which the energy requirements for rapid growth during embryonic existence are met. While still within the uterus it may be readily demonstrated that the fetus responds immediately to decrease in the oxygen supply by marked alteration in activity (11); the breathing of 4 per cent oxygen is marked by apnea of the fetus while the mother shows marked hyperpnea. The present experiments show clearly that there are available to the fetus resources for the maintenance of respiratory movements which in the adult are absent or inconspicuous.

The increased tolerance of the fetus to anoxia is of special significance in relation to the hazards of the latter part of intrauterine life including the period of expulsion from the uterus. Fluctuation in the degree of oxygenation of fetal blood as a result of increased and prolonged uterine contractions has been observed experimentally in the clinic. In so far as the magnitude and duration of uterine contractions approach the extreme state of tetanic contraction, the resources of the fetus for resistance to anoxia during labor are of obvious importance.

The hazards of parturition come to light in a species like the rabbit in which the umbilical cord is short and the length of the birth canal is many times that

of the fetal body. Despite the defense against anoxia by rabbit fetuses, the fetal mortality was observed to average about 12 per cent (12). Evidence that death was related to asphyxia was supported by findings at autopsy which indicated that the fetuses were alive at the onset of labor.

SUMMARY

The duration of respiratory movements when nitrogen was breathed instead of air was determined by the aid of individual kymograph records of respiration in 174 rabbits, 28 dogs, and 50 guinea pigs at various stages of development from the onset of viability to maturity. Analyses of arterial blood samples showed rapid development of extreme anoxemia throughout these experiments.

When placed in nitrogen immediately following birth at term, breathing continued in rabbits for 31 minutes, in dogs for 31 minutes, and in guinea pigs for 6 minutes.

In rabbits delivered operatively at the onset of viability (29 days), breathing persisted 44 minutes, and in rabbits retained within the uterus 3 days past term (35 days), breathing continued 17 minutes, which corresponded to the time of survival of rabbits suckled 3 days following birth at term. Thus, tolerance to anoxia is related to the stage of development rather than to the environment.

Furthermore, guinea pigs at birth were much less tolerant to anoxia than dogs. Although length of gestation is the same in both, the greater maturity of guinea pigs at birth is well known.

In the suckling period, survival of rabbits at 1 week was 10 minutes; at 2 weeks, 4 minutes; and at 3 weeks, 1½ minutes which is the same as that of the maternal animal. Changes in tolerance to anoxia by the dog during the suckling period parallel closely those of the rabbit.

The defense of the fetus against asphyxia is of significance in face of the increased hazard of respiratory failure during the terminal phase of intrauterine life and the early neonatal period.

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NERVE FIBER COUNTS AND MUSCLE TENSION AFTER NERVE REGENERATION IN THE RAT

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The ability of regenerating axons to branch is common knowledge. However, not all of the branches thus produced are destined to make functional connections or even to survive, and superabundant nerve sprouting does not imply correspondingly abundant peripheral innervation.

Dogliotti (1935) has based practical recommendations for improving the innervation of limbs with partial neurogenic paralysis upon the recognized ability of nerves to regenerate a larger number of branches peripherally than they had originally possessed. More recently, Aird and Naffziger (1939) have extended these experiments and critically discussed the earlier literature bearing on the problem. While substantiating the possibility of amplifying the peripheral fiber volume of a nerve, their results yet indicate that the increased nerve fiber count does not necessarily signify a commensurate functional improvement.

In none of the past nerve fiber counts has the proportion of actually reinnervated muscle fibers been determined. In order to do this, we started several years ago a systematic investigation of the problem in the rat. However, circumstances have forced several interruptions and, eventually, termination of the project in a somewhat fragmentary state.² Since facts bearing on nerve repair have gained increased practical significance, we present the results of our experiments as they stand, even though a number of points remain to be settled.

We first produced an over-all reduction of the nerve fiber complement of the sciatic nerve trunk by partial radicotomy.³ In a second operation, the sciatic nerve was cut and the two ends were reunited. The animals were examined after periods varying from seventy-four to one-hundred and eight-one days. The gastrocnemius muscles of the normal and operated sides were prepared, and tension after "maximal" stimulation of either the muscle or the sciatic nerve was recorded isometrically. A comparison of the results of direct and indirect stimulation was to furnish an estimate of the proportion of muscle fibers that had become reinnervated. Comparison with the corresponding values of the unoperated control side revealed deficits of muscle power and muscle

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² The experiments had been started in 1941 with the assistance of Dr. Donald Ross. When he left for service in the armed forces, the work was resumed in co-operation with the junior author, Dr. C. J. Campbell, who, too, had to abandon it for military duty.

³ Hines, in a recent brief report (1942), refers to his use of a similar procedure.

innervation due to the operation. After the physiological tests, some of the nerves were fixed and sectioned and fiber counts were made, as specified below.

MATERIAL AND METHODS. All operations were done on albino rats of between 200 and 300 grams body weight. The preliminary operation aimed at destroying part of the sciatic roots, L4, L5 and L6. To facilitate identification of the roots in the operation, the segmental location of a needle inserted in the sacral region of the unoperated animal was determined by x-rays and served as a landmark. In most cases, ventral roots L4 and L5 were destroyed, mostly by extradural resection. From two to seven days later, the sciatic nerve was cut in the proximal third of the thigh. The severed nerve ends were reunited by sutureless arterial tubulation, as described by Weiss (1941a, 1943).

Three types of operations were performed.

Operation A. Radicotomy on the right side with no further nerve section. Left side intact. This operation was to reveal the nerve fiber loss due to radicotomy.

Operation B. Ventral roots L4 and L5 destroyed on the right side. Sciatic nerve sectioned and reunited. Left side intact for control. This was the prevailing type of operation.

Operation C. Right side treated as in operation B. In addition, radicotomy on the left side. This operation was intended to show any superiority of terminal innervation after nerve section over the innervation from a reduced nerve not allowed to regenerate.

The kymographic records were taken in light ether anesthesia. The sciatic nerve was exposed in its full length and cut at its emergence from the lumbar plexus. All branches except the tibial were cut. The gastrocnemius muscle was connected with an isometric lever whose excursions were sufficiently linear within the range of tensions tested. All stimulations were done with the muscle under an initial resting tension of between 40 and 80 grams. The nerve was placed over platinum electrodes, supplied from the secondary coil of a Du Bois-Reymond inductorium with two dry cells in the primary circuit. The preparation was kept irrigated with Ringer's solution and the electrical tests were not started until after the effects of the ether anesthesia in the peripheral nerve had subsided. Single break shocks, as well as tetanic stimulation, were applied. For direct stimulation, the electrodes were placed on opposite ends and opposite sides of the muscle belly. All tension values referred to in this paper were obtained by slightly supramaximal stimuli.

Following the physiological tests, the nerves were fixed under stretch in Bouin's solution, sectioned, and impregnated according to Bodian's silver method. Fiber counts were made on cross sections with the aid of a camera lucida.⁴ This method was found to be correct for silver stains within about 6 per cent (Litwiller, 1938a), but in the case of young regenerated nerves containing large numbers of fine fibers, the error may well be assumed to be nearer to 10 per cent.

⁴ Counts were made with the assistance of Dr. Raymond Litwiller.

Of one hundred and twenty operated animals, only sixteen have come through to the terminal tests.

Fiber counts. The purpose of the radicotomy was largely defeated by the prompt regeneration of the severed roots. Sections through proximal levels of the sciatic nerve show numerous regenerating fibers, frequently in strands, interspersed with the old fibers of the nerve. In eight cases subjected to operations A or B, listed in table 1, the total of nerve fibers counted in the proximal sciatic on the partially radicotomized side, is 43,802, while the corresponding total for the unoperated side is 43,167. One must bear in mind, however, that the transected root fibers had to regenerate for a distance of approximately 30 mm. before arriving at the level of the distal nerve section. Assuming conservatively a rate of advance of between 2 and 3 mm. per day (Gutmann et al., 1942), it would have required some two to three weeks for the fibers to span this

TABLE 1

Nerve fiber counts at standard sampling levels of experimental and control sciatic nerves

CASE	OPERATION	LEFT (CONTROL) SIDE		RIGHT (EXPERIMENTAL) SIDE			
		Proximal nerve	Distal nerve	Proximal nerve	"Gap" region nerve	Distal nerve	Regeneration index $r = \frac{100n_{\text{dist}}}{n_{\text{prox}}}$
D4	A	5605	5495	4790		5400	
D5	B	5850		5860	2576	2595	44
D10	B	5350	4935	7505*		5655	76
D12	B	5895		5235	4245	3218	61
D15	B	5385		5590	5545	4695	84
D18	B	4572		5460		3245	62
D37	B	5165		4613	4770	3425	71
D38	B	5345		4435	4855	4075	92
E1	C	4655	5525	5415	5240	5210	96

* Apparent overcount due to overstained preparation.

distance. Thus those sciatic fibers belonging to the undamaged roots should have had ample time to occupy all available peripheral pathways prior to the arrival of the second growth of root fibers.

Table 1 lists sample nerve fiber counts. On the right side, which, except for case D4, is the one on which the sciatic nerve had been transected and allowed to regenerate, counts are reproduced at three levels: normal level far proximal to the nerve union; a level well inside the old peripheral stump, with the counts of all branches of the tibial and peroneal nerves combined in a single figure; and through the so-called "gap" region, which is the level of transition from the proximal to the distal stump.

In none of the cases pertinent to the problem, i.e., series B and C, has there been an increase in the number of fibers distal to the nerve union over the number found in the proximal stump, such as described, for instance, by Greenman (1913). The last column of table 1 gives the ratio of number of nerve fibers

counted distally to the nerve union over that counted proximally in per cent values. The distal deficit ranges from 4 to 56 per cent. In half of the cases, the peripheral nerve has been filled up to more than 75 per cent. In case D5, the deficit is already present in the gap; in case D37, it appears beyond the gap; in case D12, the gap yields a count intermediate between the proximal and peripheral stumps; and in case D38, there is even a slight increase in the gap region, preceding the peripheral decline. The peripheral fiber deficit is not due to insufficient time allowed to the regeneration. If percentage regeneration is plotted against time elapsed after transection, provided the latter is more than two months, the points scatter at random (fig. 1). For example, case E1 shows a deficit of only 4 per cent at seventy-four days, while case D18 shows a 38 per cent deficit as late as one-hundred and sixty-six days after the operation. It is

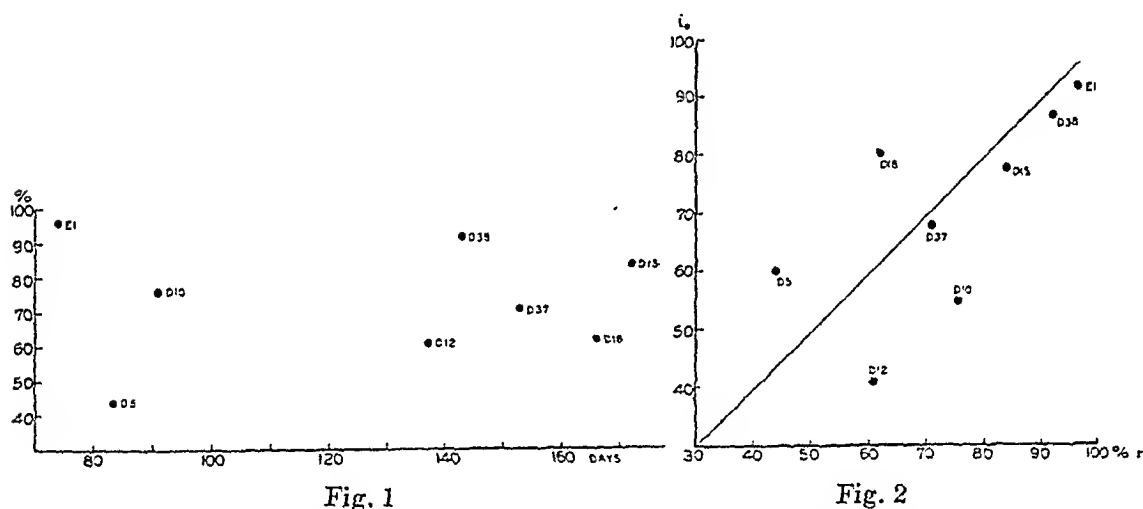


Fig. 1

Fig. 2

Fig. 1. Nerve fiber count in the regenerated distal portion of the nerve, expressed in per cent of the fiber count in the old proximal portion, at various periods after nerve section.

Fig. 2. Relation between volume of nerve fiber regeneration and proportion of reinnervated muscle fibers. Abscissae: Regeneration index (r , table 1). Ordinates: Innervation index (i_0 , table 2).

safe, therefore, to conclude that the observed distal deficit is permanent, particularly in cases D12, D37 and D18, with more than four months' regeneration time.

Some clue as to the cause of the peripheral fiber deficit has been found in the histological examinations. Details of the course of regeneration in nerves united by arterial sleeves have been presented elsewhere (Weiss, 1943). It was described there that this method precludes the profuse branching commonly found in the scar region at the suture line. Under ideal conditions, therefore, the number of fibers in the gap may be expected to equal the number of fibers in the proximal stump, and to continue without essential change into the peripheral stump. Fibers deflected or arrested in their advance constitute a permanent loss to the peripheral nerve, and statistically, there has been more loss than was compensated by sporadic ramifications. Actually, in all three cases with a major peripheral nerve deficit, i.e., D5, D12 and D18, the fiber loss could

be traced to faultiness of the arterial link. As described previously (Weiss, 1943), any leak in the arterial wall may cause appreciable fiber escape. In cases D5 and D12, a stream of nerve fibers has escaped through a leak in the arterial sleeve. In case D18, the peripheral deficit can be traced to excessive width of the arterial sleeve around the peripheral stump, which has permitted part of the nerve fibers to escape through the open space. The important fact about these cases is the consistent lack of spontaneous compensatory amplification of the regenerating fiber volume by peripheral branching.

All our observations on the return of motility conform with the description given in an earlier paper (Weiss, 1943). Briefly, as motility returns, it involves essentially co-contraction of the dorsi-flexors and plantar flexors of the foot, with the latter prevailing owing to their greater bulk. The result was mostly rigid extension of the foot with flexion of the toes. There were indications of gradual ankylosis of the ankle, attributable to the lack of antagonistic joint movements.

The failure of co-ordinated movements to return after regeneration of a divided sciatic nerve seems to be due to the random regeneration of the nerve fibers, in conjunction with the inability of the rat central nervous system to make any re-educative adjustments of the functional disorder created by the anatomical disarrangement of the central-peripheral connections (Sperry, 1941, 1943). Some of our animals exhibited, however, a trace of reciprocal ankle movements superimposed upon a more conspicuous background of rigid extension.

In spite of their irrelevance for locomotion, the reinnervated muscles, after considerable initial atrophy, have regained appreciable power. For the degenerative phase and general recuperation of muscle power after nerve section in the rat, see Hines, Thomson and Lazere (1942).

Physiological tests. Stimulation thresholds were, on the whole, higher for the regenerated than for the control nerves, which may be ascribed to the heavier connective tissue sheath around the former. A comparison between the isometric tensions obtained from gastrocnemius muscles after "direct" and after "indirect" sciatic nerve stimulation, as well as between these and the ones obtained from the opposite (control) side, is presented in percentage values in table 2.

The ratio of maximal tensions recorded after indirect and direct stimulation expresses the degree of functional reinnervation of the muscle. A maximal stimulus to the muscle directly sets all contractile elements in operation. A maximal stimulus to the nerve activates all innervated muscle fibers. Accordingly, any excess of tension obtained on direct stimulation over that obtainable by indirect stimulation can be ascribed to muscle fibers which have failed to receive innervation. This would be strictly valid only if the average sizes of innervated and uninnervated muscle units are statistically the same. If the innervated units should prove to be, on the average, larger and, therefore, stronger, an empirical correction factor would have to be introduced.

With this qualification in mind, the ratio of "indirect" over "direct" tension may be used as index of the proportion of innervated to total number of muscle

fibers. This ratio, expressed in per cent values, will be referred to as the innervation index (i). The third column of table 2 reveals that the innervation index of normal control muscles (i_c) is mostly well under 100 per cent. This means that even in a normal muscle a varying fraction of fibers cannot be stimulated through the nerve. The average innervation deficit for the normal control muscles in the thirteen cases of series A and B is 7.5 per cent. This compares well with a deficit of 8.3 per cent computed from values given for seven control gastrocnemius muscles by Hines, Thomson and Lazere (1942, table 1). The most plausible explanation of this deficit would lie in injury suffered by some nerve fibers during the manipulation; another few fibers may be in the process of degeneration, constantly proceeding in nerves (less than 1 per cent according to Duncan, 1930). Some others may have undergone

TABLE 2

SPECIMEN	OPERATION	INNervation INDEX (i)		MUSCLE POWER ON OPERATED SIDE IN PER CENT OF CONTROL SIDE	INNervation QUOTA ON OPERATED SIDE IN PER CENT OF CONTROL SIDE
		Left (control) side	Right (experi- mental) side		
		i_c	i_o	m	n
D4	A	84.5	78.5	79.0	73.5
D6	A	84.2	50.0	46.5	27.5
D8	A	100.0	73.5	143.5	105.7
D2	B	92.5	31.1	31.2	10.5
D5	B	89.5	60.0	41.5	27.6
D7	B	92.5	60.3	89.2	58.2
D10	B	95.0	54.7	53.2	30.6
D12	B	93.1	40.9	21.8	9.5
D15	B	100.0	77.7	85.0	66.0
D18	B	92.0	80.5	108.0	94.5
D25	B	92.5	78.0	68.8	58.2
D37	B	92.5	67.8	93.5	68.5
D38	B	95.2	87.0	120.5	110.0
E1	C	90.6	91.5	92.1	93.1
E2	C	45.0			100.0

degeneration as a result of the lesion of the symmetrical nerve of the opposite side. Contralateral effects of this kind have been described (Greenman, 1913; Tamaki, 1933), but seem to subside with increasing age after the operation (Tamaki, 1936).

In contrast to a deficit on the control side of 7.5 per cent, the deficit on the experimental side is much greater, the average of thirteen cases being 36 per cent. This means that, on an average, fewer than two-thirds of the fibers of the gastrocnemius have received reinnervation.

Besides being less completely innervated, the experimental gastrocnemius is also weaker than its control. The power ratio between the experimental gastrocnemius and its opposite control is given in the fifth column (m) of table 2. The figures represent maximum tension of the experimental muscle expressed in percentage of the maximum tension obtainable from the normal opposite

muscles. While in three cases (D8, D18, D38) the reinnervated muscle has obviously overshoot its normal partner, all other cases show reduced power in the reinnervated muscle. How the loss is to be apportioned between atrophy and degeneration, cannot be determined from these figures.

A comparison between the trend of the innervation index and the muscular power index points toward a continued increase in muscle power beyond the attainment of the final innervation ratio. While in none of four cases examined less than one-hundred forty days after the operation, the muscle index exceeds the innervation index, it surpasses it appreciably in all but one of six cases examined later than one-hundred forty days. This would indicate that the non-innervated fibers of a partially reinnervated muscle continue to gain, possibly benefitting from the passive exercise to which they are subjected by their innervated partners.

The general result of these tests is that in most cases a certain fraction of the denervated muscle fibers have remained without reinnervation.

Nerve fiber counts through regenerated nerves could furnish an accurate measure of the degree of peripheral reinnervation only if the average size of the motor units should prove to be the same before and after regeneration, since the effectiveness of muscle reinnervation is determined both by the number of regenerated motor neurons and the extent of their intra-muscular branching. Little attention has been given to this point in the past. In this connection the following data deserve mention.

In figure 2, the innervation indices (i_0) of the experimental muscles (table 2) have been plotted against the fiber regeneration indices (r), as listed in table 1. The graph thus correlates the proportion of reinnervated muscle fibers of a given muscle with the proportion of regenerated nerve fibers counted in the peripheral nerve stump. There is, on the whole, correspondence among the two values, as is expressed in their scattering about a straight line inclined under a 45° angle. Four cases, D15, D37, D38 and E1, conform very closely, indicating that the size of the motor units was nearly the same before and after regeneration. In contrast, cases D5 and D18 show an unexpectedly high, and cases D10 and D12 an unexpectedly low, innervation index for the number of fibers actually counted in the nerve trunk. In this connection, misregeneration of sensory fibers into muscles must be taken into account. Functional tests of such fibers have shown them to be much inferior to motor fibers in establishing transmissive connections (Weiss, 1934, 1935). Consequently, even if the fiber mass of the nerve is distributed proportionally over the periphery, the number of functionally effective efferent fibers in any one muscle might be smaller than extrapolation from the total fiber count would indicate.

The lesson of these results is that the number of nerve fibers counted in the peripheral nerve provides no reliable index of the degree of reinnervation attained in the muscle, although statistically the two factors are correlated. This conclusion agrees with the view expressed by Aird and Naffziger (1939). Consideration of the manner in which terminal reinnervation occurs can readily account for the observed variability. If a nerve fiber arrives in the muscle

inside of an old degenerated Schwann tube, it is likely to reoccupy all the terminal ramifications of this tube. Thus the original pattern of innervation will be retraced and that motor unit will be restored to its original size. If some of the old branches have become obliterated or obstructed, these would be missed by the regenerating fiber tips, and a corresponding reduction of the size of the motor units would ensue, as, for instance, in cases D10 and D12. Excessive terminal ramifications, on the other hand, may be expected in nerve fibers which have taken extratubal courses and arrived in the muscle without the benefit of an old branching pattern as guide. Such fibers may stray about and innervate any number of not yet innervated muscle fibers. Earlier studies in amphibians have shown that when nerves are made to enter a muscle by paths other than the original portals of the motor points, their branches tend to pervade the muscle profusely (Fort, 1940). If this should hold for mammals, too, a fairly complete reinnervation of all muscle fibers, even from a small number of reinnervating extratubal nerve fibers, might be expected. The fact that this has not occurred in the majority of our cases seems to prove that most of the regenerating fibers have come by intratubal courses. Being thus trapped in the old innervation beds, they had no opportunity for a compensatory expansion within the muscle.

DISCUSSION. The facts reported in this paper underscore the complexity of the quantitative aspects of nerve regeneration. Factors bearing on the volume of peripheral innervation are, in proximo-distal, as well as chronological, progression: 1, number of fibers in the proximal nerve stump; 2, incidence of branching among the regenerating sprouts; 3, number of fiber branches entering or by-passing the peripheral nerve stump; 4, proportion of fibers surviving subsequent resorption; 5, number of fibers establishing effective transmissive peripheral connections; 6, number of intramuscular terminal ramifications (size of the "motor unit"); 7, degree of muscular atrophy and degeneration during the denervated period, and of muscular recovery after reinnervation.

1. Proximal fiber source. The number of neurons assigned to any one given peripheral area is fairly constant among members of the same species. Embryonic factors affecting this nerve quota have been extensively studied (review: Detwiler, 1936). Once established, it fails to respond to demands for additional peripheral innervation. Adult spinal centers do not send out new nerve fibers into districts experimentally overloaded with tissues requiring innervation. Consequently, saturation with nerve fibers of an enlarged periphery could be effected only by increased peripheral branching of the existing neurons (Weiss, 1937). Conversely, a tissue mass of subnormal size, when confronted with a normal nerve source, fails to absorb the total available nerve supply, and accepts nerve fibers only in proportion to its actual mass (Weiss and Walker, 1934; Litwiler, 1938b). Saturation density reached, the supernumerary nerve fibers remain unconnected. The final fate of such fibers is not known.

These facts have led to the realization that the volume of peripheral innervation is not primarily determined by the size of the nerve fiber source (Weiss, 1941b). The peripheral tissues themselves exert a decisive control over the

density of their innervation by regulating the admission quota, though not the production quota. Unless branching is enhanced by special means, an undersized nerve source is bound to remain undersized even in the face of greater peripheral demand.

2. *Degree of branching.* Brief discussions of the mechanism of nerve branching have been presented on earlier occasions (Weiss, 1934b, 1941b). The facts do not support the notion that regenerating nerve fibers branch from intrinsic causes as do trees. Nerve fibers branch only in response to inhomogeneities in their environment. The intensity of branching is, therefore, a function of the incidence of both gross mechanical obstacles and submicroscopical disorientations of the contact substrata along which the nerve fiber proceeds. The growth energy of the regenerating fiber must also be considered in that branches which would have remained abortive in weakly growing fibers might assert themselves in a more vigorously growing fiber. In conclusion, the number of branches formed is determined by the local factors of the course, rather than the distal factors of the destination, of the nerve fiber.

In this fact lies the explanation of the failure of undersized nerve sources in our experiments to provide full peripheral reinnervation. A loss of fibers through leaks in the splicing sleeve was never compensated by increased branching prior to, or after, entry into the peripheral stump. The reason for this lies in the mechanics of the tubular splice, which precludes the formation of a disoriented scar (Weiss, 1943). We might have obtained a better filling of the peripheral stump if the condition at the suture line had been less orderly. The same argument applies to the observation of Hines (1942) that regeneration following the crushing of the tibial nerve does not make up for a reduction of the fiber complement of this nerve produced by partial radicotomy. Unlike nerve section, mere crushing often fails to rupture the neurilemmal tubes, hence fails to provoke the extensive branching characteristic of a messy scar. Consequently, neither our own nor Hines' negative results are necessarily at variance with the claim of the earlier authors that an effective peripheral amplification of nerve volume by branching in the scar is feasible. For practical purposes, it should be remembered, however, that any merit the scar may have in causing branching, is nullified by the increased resistance it offers to the further advance of the multitudinous branches.

3. *Neurotization of peripheral nerve stump.* What proportion of the nerve fibers present in the gap will actually enter the distal nerve stump, seems to be entirely a matter of chance. The hypothesis of a chemotropic direction of nerve fibers towards the distal stump (Cajal, 1893; Forssman, 1900), occasionally disputed in the past (Dustin, 1910), has been found untenable in view of experimental evidence (Weiss, 1934b, 1941b). Nerve orientation is determined wholly by the pathway structure of the scar. Moreover, strands of sheath cells, growing proximad into the scar, may serve as traps for nerve fiber sprouts (Young, 1942). Similar traps are constituted by the degenerated Schwann tubes of the peripheral stump. However, since, as a rule, more than one regenerating nerve fiber may enter into a tube (Boeke, 1921), the number of tubes actually invaded is no measure of the density of reinnervation of the peripheral stump. While some of

the old tubes become overpopulated, others remain abandoned. Nevertheless, the total number of fibers in the distal nerve stump approximates its normal capacity, regardless of the size of the proximal supply (Weiss and Cummings, 1943).

4. *Maturation of peripheral stump.* Of the fiber branches entering the peripheral nerve stump, not all are destined to survive and mature. But neither the criteria nor the mechanism of secondary fiber branch elimination are known (cf. Young, 1942). Spatial crowding within undistensible tubes, metabolic competition among the several branches supplied from a common nerve cell, and presumably effects extending into the nerve fiber from its terminal organ are factors which will have to be considered in this connection. Their operation may remove much of the initial overproduction caused by the branching in the scar (cf. Bocke, 1921).

5. *Neuro-muscular connections.* The terminal branching pattern of the regenerated motor neuron depends on the channels through which the nerve fibers arrive, as well as on the condition of the muscle fibers. Nerve fibers regenerating inside of an old Schwann tube find a preformed pattern of arborization. Extratubal fibers will follow irregular courses. As stated above, there are indications that this latter category is not prevalent.

Innervation of a single muscle fiber by several nerve fibers may occur on occasions, but is functionally irrelevant. Its regular occurrence is prevented by a protective reaction which renders the innervated muscle fiber immune to impregnation by further nerve branches (cf. Fort, 1940). Attempts at so-called "hyperneurotization" by forcing more than one nerve ending upon a muscle fiber (Erlacher, 1914) are based on questionable physiological premises and have no practical significance.

This brief survey, incomplete as it is, may yet give an idea of the intricacies of the problem of quantitative recovery in nerve regeneration. It makes it obvious that the mere fact of an increased fiber count below the suture line of a peripheral nerve is no sufficient guarantee of functional enhancement. This is not to say that under certain conditions practical benefits may not come from inducing peripheral nerve fibers to divide by making them regenerate. Our data merely caution against accepting such peripheral amplification as either the sole, or even a pertinent, gauge of terminal improvement.

SUMMARY

In an attempt to determine whether the volume of fiber regeneration after nerve transection is indicative of the degree of recovery of power in the reinnervated muscles, nerve fiber counts of regenerated sciatic nerves were compared with measurements of isometric tension produced by the gastrocnemius muscle after "direct" and "indirect" stimulation with "maximal" shocks. The following conclusions have been reached.

There is no constant relation between the number of fibers growing from the proximal stump of the nerve and the number found in the peripheral stump (table 1). Spontaneous regulatory axon branching to compensate for fiber deficits does not occur. Profuse branching may, however, be evoked by scar

tissue at the suture line. Yet, even this does not of itself insure that the volume of nerve fibers reaching the muscles will be correspondingly amplified.

From 9 to 69 per cent of the muscle fibers of the reinnervated muscles have failed to receive functional reinnervation (table 2). These deficits can be correlated with fiber deficits in the peripheral nerve trunk only very grossly (fig. 2). In the individual case, the discrepancy may be great. For instance, a quota of only 62 per cent regenerated fibers in the nerve may reinnervate as many as 80 per cent of the muscle fibers (D18), while, conversely, nerve regeneration of as much as 76 per cent may provide only 55 per cent reinnervation (D10).

Factors affecting the density and functional effectiveness of regenerative muscle reinnervation are 1, number of fibers in the proximal nerve stump; 2, axon branching in the scar; 3, fiber admission into the peripheral stump; 4, secondary resorption of fiber branches; 5, proportion of fibers effecting myoneural junctions; 6, extent of intramuscular axon branching; 7, condition of the muscle fibers. This variety explains why regenerative success is not solely predicated on the intensity of fiber proliferation at the suture line.

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PURIFICATION AND BIOASSAY OF TISSUE EXTRACTS CAPABLE OF LOWERING THE BLOOD PRESSURE OF HYPERTENSIVE RATS

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Evidence that hypertension following renal arteriolar constriction could be prevented by the presence of sufficient normal kidney tissue (1-3) led to the extraction of normal kidneys for a material which would lower the elevated blood pressure level of experimental hypertensive animals or of patients with hypertension (4-10). Encouraging results were obtained by several groups of investigators with a relatively crude protein extract from hog kidney. The attractive theory was developed that such an extract contained an enzyme which destroyed the humoral pressor agent of the ischemic kidney, presumably angiotonin (11, 12) or hypertensin (13). It has not been clearly demonstrated, however, that the pressure lowering response to extract injections was due to a specific inactivation of a pressor material, and not to a more general reaction to a foreign protein (10). Further, it now seems clear that the active materials contained in extracts prepared in different laboratories are not chemically identical.

We have attempted a purification of the active protein component contained in hog renal extracts, directed toward a clinical testing of the material. Before attempting a chemical fractionation, it seemed imperative that a reproducible and standardized method of bioassay be constructed. Toward this end we have made a study of the pressure lowering response evoked in the hypertensive rat.

Hypertension in the test rats was induced by the simple operative procedure of wrapping both kidneys with cellophane at a single operation (14). The incidence of hypertension has not been satisfactory, but, for some unknown reason, high blood pressure levels have been obtained even less consistently when various samples of either silk or rayon were substituted for cellophane. Of some 1600 rats subjected to cellophane kidney wrapping, 37 per cent died before the 30th day after operation, when routine pressure recordings were started. A full 33 per cent more showed no tendency to develop definitely hypertensive pressure levels within 60 to 90 days, and were discarded. Even with the 480 rats which showed definite hypertension, only 320 were entirely satisfactory for assay work.

Before any assurance could be placed upon a pressure fall which followed treatment in the hypertensive rat, it was necessary to form some estimate both of the extent of daily fluctuation of the pressure level which might be expected in a non-injected control, and, more particularly, of the greatest pressure decline which might reasonably be expected to occur spontaneously. Daily pressure recordings, by the technique of Williams, Harrison and Grollman (15), were therefore made on about 100 hypertensive rats for a period of 20 to 30 days. As

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might be anticipated, the higher the blood pressure level, the greater was the fluctuation. Only the decline below the average pressure level was analyzed as pertinent to the problem. Statistical treatment showed that the greatest pressure reduction reached at any time during the test period, X , was related to the average pressure level for the period, Y , according to the formula $Y^2 = 532X + 8,500$. The corresponding curve is shown in figure 1. If the pressure reduction following extract treatment is to be considered significant, it is but reasonable to demand that it be greater than this average spontaneous fall.

In preliminary experiments to determine the best route and the time duration over which the kidney extract was to be administered, it was found that the

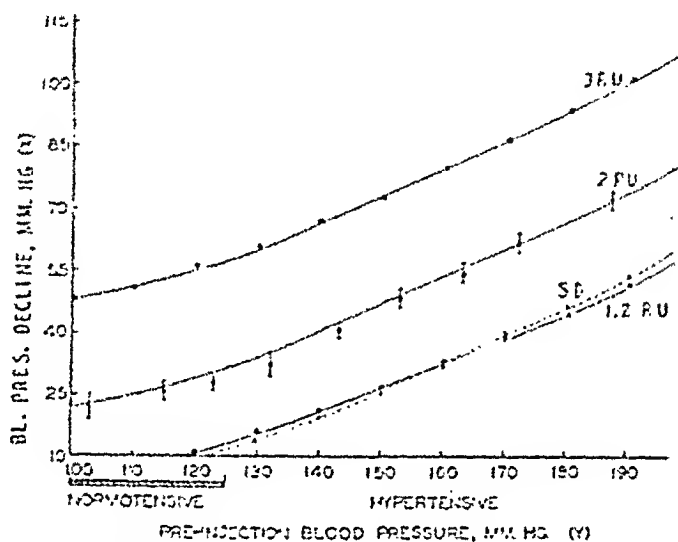


Fig. 1. Correlation between the blood pressure decline produced in hypertensive rats by the injection of hog kidney extract and the pre-injection pressure level of the test rat.

2 R.U., a 2 rat unit response, $Y^2 = 484X - 167$. Vertical lines represent probable errors. Sixteen rats in each group.

3 R.U., the upper critical level of reproducible responses, $Y^2 = 484X - 13,148$.

1.2 R.U., the lower critical limit of reproducible responses, $Y^2 = 484X + 9,863$.

S.D., (dotted line) the average lower limit of spontaneous pressure decline shown by uninjected control hypertensive rats, $Y^2 = 532X + 8,500$.

most consistent pressure reduction responses were obtained after 4 days of twice daily injections. A volume of 0.25 cc. was given at each injection, so that each rat received a total of 2 cc. If the potency of a given extract proved low, it was concentrated for a second assay, for any attempt to retest at a greater injection volume never gave satisfactory results. Intramuscular injections produced a response $1.3X$ greater than did subcutaneous, and $3X$ greater than did intraperitoneal injections.

Typical blood pressure changes which follow the injection of an active kidney extract are shown in figure 2. Pressure recordings were made at the same time each morning, before the extract injection. The blood pressure usually started to decline from the pre-injection level on the 2nd or 3rd day of the test, reached its lowest level on the 4th or 5th day, rose sharply to the pre-injection level or

slightly below, and then showed a secondary decline which persisted for a variable period. Almost coincident with the time interval over which the rat showed a pressure level below that of the pre-experimental period, the animal proved wholly or partially refractory to a second injection series (fig. 2). This refractory period is not necessarily related to the pressure level, however, for an extract too weak to elicit an appreciable pressure reduction may render the rat insensitive to a second injection series (after 7–10 days) with a highly potent extract. The slow recovery of the pressure to sustained high (pre-injection) pressure levels after cessation of injections, and the development of the refractory state, have been observed with both crude and more purified extracts.

The assay itself was based on the first part of the response only, i.e., the initial pressure reduction and the rapid return to pre-injection levels. To allow ample time for recovery of sensitivity of the test animal, a period of 21 days was allowed between successive assay tests. After an animal had been used for 4 to 7 tests,

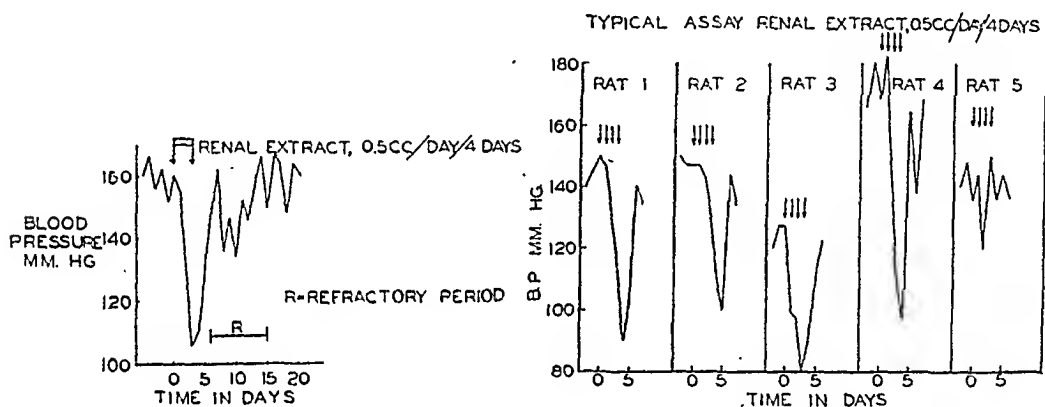


Fig. 2. A typical blood pressure response of a hypertensive rat to 4 twice daily injections of renal extract, and a typical assay based on the best 4 of 5 responses to a given extract. Rat 5 was discarded as showing refractoriness to the extract.

it usually became partially or completely refractory even after the 21 day period, and was discarded.

Harrison and associates (16) have pointed out that the actual pressure reduction obtainable with renal extract administration depends upon the initial pressure level of the hypertensive animal. Since a given group of rats was composed of individuals with different degrees of hypertension, we attempted a quantitative measure of the correlation between absolute pressure reduction and the pre-injection level. The pressure reductions obtained with a series of comparable extracts given, at the same dosage level, to 160 rats, were therefore grouped according to the pre-injection pressure levels, obtained by averaging the daily recordings taken on the 3 successive days before the start of the test. To obtain the actual pressure reduction in millimeters Hg, the two lowest pressure levels reached during the first 6 days of the test period, or, in the case of a somewhat aberrant response, the pressure levels on the 4th and 5th days, were averaged. The relation of the average pressure reduction for each group of rats, X , to the

average pre-injection level, Y , was found to follow the curve $Y^2 = 484X - 167$ (fig. 1). Hence the higher the hypertensive pressure level, the greater the decline following extract treatment. Extrapolation of the derived curve would indicate that a measurable response should be obtainable with rats showing pressures in the "normotensive" range. This seemed unreasonable in view of the fact, recognized by others as well, that consistent pressure changes would not follow extract injections in normal control animals. When rats which had been subjected to the kidney wrapping, but which had not developed definite hypertension, were similarly treated, however, about half of them did show a significant pressure reduction (fig. 1). An accurate assay could actually be made on such normotensive animals, although, since the results were more difficult to judge accurately, no more than 2 were ever included in a given 5 rat series.

Some clue as to whether a given normotensive rat was in reality an "incipient hypertensive", and would respond to extract treatment, could often be gained from a study of the daily fluctuation of previous blood pressure recordings. Curiously enough, once the pressure had been lowered below normal by extract treatment, it returned, in many cases, not to the pre-injection level but well beyond into hypertensive ranges. These incipient hypertensive rats showed fibrocollagenous hull formation around at least 1 kidney.

Roughly 20 per cent of all animals chosen for assay can be expected to show atypical responses: they may be completely resistant to the extract, or they may show peculiar pressure responses, such as a precipitous decline on the 1st day of the injection series; others failed to show a complete restoration of pressure level. It was found convenient, therefore, to use 5 rats as a test unit, with the best 4 responses, none of which was atypical, averaged to determine the response to the extract tested.

To establish a basic unit of measurement, the average pre-injection pressure level for the 160 rats used in the above study (158 mm. Hg) was taken as the standard, and the average pressure reduction (52 mm. Hg) assigned the value of 2 rat units. Since 2 cc. of extract was given each rat, the response also represented 1 R.U. per cc. extract. A 2 R.U. response for an animal with pre-injection pressure level other than 158 mm. Hg was determined by reference to the curve shown in figure 1. The average pressure of unoperated control animals was found to be 100 ± 2 mm. Hg. A hypertensive pressure level of 158 mm. Hg therefore represented a pressure elevation of 58 mm. Hg over the normal.

Before this rat unit could be used as a quantitative measure of the activity contained in a given extract, it was necessary to establish a linearity between dosage given and response obtained. An extract which contained 1 R.U. per cc. was diluted or concentrated to make a series of fractions of known strength. As shown in table 1, good correlation between the dosage and response were found between the limits of 1.2 and 3.0 R.U. In other words, between these limits, 0.1 R.U. was equal to a pressure reduction of 2.6 mm. Hg in an animal with pre-injection pressure level of 158 mm. Hg. Statistical analyses of a number of assays indicates that a difference of 0.2 R.U. between 2 extracts is required for probable significance, and one of 0.3 R.U. for clear significance.

For purpose of definition, then, 1 rat unit is the amount of material which, when given intramuscularly twice daily for a period of 4 days, will lower the blood pressure of 4 of 5 hypertensive rats from a 3 day average pre-injection level of 158 mm. Hg by an average of 26 mm. Hg, such reduction to be reached within 6 days of the beginning of the injections, be maintained for at least 2 days, and be followed by a full pressure return to the pre-injection level. For standard assay work, all extracts were prepared so that 1 cc. was equivalent to 200 grams frozen tissue.

Chemical purification of the active material contained in kidney extracts. An extraction of the pressure reducing material contained in kidney extracts is made peculiarly difficult because the extremely low yield necessitates the use of large quantities of tissue, and the handling of bulky precipitates and slow filtrations. Although some 4 tons of hog kidneys were processed for this study, many points have not been investigated as thoroughly as might be desired.

TABLE 1
Blood pressure response to the injection of kidney extracts of known potency.

FRACTION	NUMBER OF RATS TESTED	EXPECTED ACTIVITY, R.U.	DETERMINED ACTIVITY, R.U.	DIFFERENCE, R.U.
Standard	5		2.0	
A	5	0.8	0.2 (?)	0.6
B	4	1.2	0.9	0.3
C	3	1.4	1.3	0.1
D	5	1.6	1.5	0.1
E	5	2.4	2.6	0.2
F	4	2.8	2.7	0.1
G	4	3.0	2.8	0.2
H	5	3.2	2.9	0.3
I	3	3.6	2.9	0.7

The first extraction procedures (steps 1-3, table 2) were patterned after those successfully used by Jensen and associates (7). The extract obtained, while active, was dark, highly viscous, and often produced local reactions at the injection sites. The next purification step was based on the fact that the active material is precipitated between 25 and 50 per cent acetone concentration. It was later found, however, that as much as 20 per cent of the original activity could be lost by inclusion with the bulky inactive precipitate formed at 25 per cent acetone concentration, and the step was omitted from the procedure. The use of acetone has been retained in the preparation of large amounts of extract for clinical use, simply as a convenient means of concentration. After completion of the dialysis (step 8), the albumin fraction which was precipitated between 25 and 80 per cent acetone concentration was dissolved in a small volume of water, thus circumventing the necessity of a prolonged concentration by *in vacuo* distillation.

The fractionation at pH 2 (step 5) has proved to be a simple method for rapidly reducing the solid content without an appreciable loss in activity. The addition of acid changes the solubility of many of the inactive proteins so that

they no longer dissolve in the same volume of water. Since activity is gradually destroyed at the low pH level, the time interval prescribed must be adhered to, and the later filtrations done rapidly, which makes the step unsuited to the preparation of large amounts of extract.

Earlier crude extracts often proved toxic to the test animals. The reactions seem to have been due to inactive globulins, which can be eliminated by either

TABLE 2

Extraction and purification of the material contained in kidney extracts capable of lowering the blood pressure of hypertensive rats

STEP	SOLID CONTENT GRAMS	ACTIVITY R.U.	PROCEDURE*
1			100 lbs. (45 kgm.) frozen kidneys finely ground into 100 L. 0.1 sat. $(\text{NH}_4)_2\text{SO}_4$ soln., stirred 4 hrs. 1:5 HCl added to pH 3, stirred 2 hrs., filtered, residue discarded†
2	130	300	Add $(\text{NH}_4)_2\text{SO}_4$ to 0.7 sat., ppt. dissolved in 5 L. water. Add $(\text{NH}_4)_2\text{SO}_4$ to 0.2 sat., ppt. discarded. Add $(\text{NH}_4)_2\text{SO}_4$ to 0.6 sat., ppt. dissolved in 2 L. water
3	74	270	Add $(\text{NH}_4)_2\text{SO}_4$ to 0.25 sat., ppt. discarded. Add $(\text{NH}_4)_2\text{SO}_4$ to 0.6 sat., ppt. dissolved in 350 cc. water
4	48	242	Add cold acetone to 25%, ppt. discarded. Add acetone to 80%, ppt. dissolved in 225 cc. water
5	38	204	Add 1:5 HCl to pH 2, stand 1 hr., add $(\text{NH}_4)_2\text{SO}_4$ to 0.5 sat., ppt. suspended in 225 cc. N/10 acetate buffer, pH 4.8, stirred $\frac{1}{2}$ hr., filtered. Insol. ppt. discarded
6	13	203	Add $(\text{NH}_4)_2\text{SO}_4$ to 0.45 sat., ppt. discarded. Add $(\text{NH}_4)_2\text{SO}_4$ to 0.6 sat., ppt. dissolved in 225 cc. water
7	5.5	155	Add $(\text{NH}_4)_2\text{SO}_4$ to 0.45 sat., ppt. discarded. Add $(\text{NH}_4)_2\text{SO}_4$ to 0.55 sat., ppt. dissolved in 225 cc. water
8	1.7	143	Dialyzed against distilled water, conc. to 225 cc. <i>in vacuo</i> , add merthiolate 1:20,000, NaCl to 0.8%, filtered through Seitz filter
	1.6	142	Final extract, 1 cc. equivalent 200 grams kidney, solids 7 mgm./cc., activity 0.63 R.U./cc. Non toxic

* All procedures done in cold.

† A re-extraction of the meat residue with 50 L. 0.1 saturated ammonium sulfate solution yields only 45 R.U. additional activity.

step 5 or 6 (table 2). The final extract produced no untoward symptoms in either normal or hypertensive rats.

Extract prepared in large quantities has been of two types: *a*, prepared by steps 1 to 5 and 8, having a final solid content of 60 mgm. per 200 grams kidney, and 0.9 R.U. activity per 200 grams kidney; *b*, prepared by steps 1 to 3, 6, 8 and 4, with final solid content of 40 mgm. and activity of 0.75 R.U. per 200 grams kidney.

Present indications suggest that there is but a single active component in this protein extract, apparently an albumin, precipitated between 0.45 and 0.55 saturation with ammonium sulfate, and heat labile. The activity lost in the extraction procedures can be reclaimed by collecting the filtrates after several

successive precipitations at 0.45 saturation with ammonium sulfate. The extract loses complete activity when allowed to stand for 4 months at room temperature, and $\frac{1}{3}$ its activity over the same period in the cold.

Specificity of the pressure lowering activity of kidney extracts. The recovery of activity throughout the fractionation procedure, and the progressive concentration of the active material by the discard of inactive proteins, indicates strongly that the decline in pressure evoked cannot be dismissed simply as being due to a general reaction to a foreign protein. The test animals showed no symptoms of anaphylactic shock, and suffered no change in body temperature. Other com-

TABLE 3

Specificity of the blood pressure reducing response obtained in hypertensive rats

MATERIAL TESTED	SOLIDS INJECTED, MGM.	ACTIVITY		
		R.U.	R.U./GM. SOLIDS	R.U./KGM. TISSUE
Hog kidney, crude extract.....	400	2.0	5.0	5.0
Hog kidney, purified extract.....	120	2.0	16.7	5.0
Hog kidney, purified globulin fraction.....	80	0		
Hog kidney, purified albumin fraction.....	15	1.4	93.3	3.5
Hog spleen, purified extract.....	145	1.7	11.8	4.2
Hog spleen, purified globulin fraction.....	70	0.3	4.3	0.7
Hog spleen, purified albumin fraction.....	30	1.2	40.0	3.0
Hog muscle, purified extract.....	68	1.4	20.6	3.5
Hog muscle, purified albumin fraction.....	20	1.0	50.0	2.5
Hog blood, globulin fraction.....	135	0		
Hog blood, albumin fraction.....	48	0.6	12.5	1.5
Horse serum, unfractionated.....	160	1.5	9.3	750
Horse serum, globulin fraction.....	48	0.6	12.5	16.5
Horse serum, albumin fraction.....	0.8	2.0	2500	250
Egg albumin.....	124	0		
Pepsin.....	100	0		
Diphtheria antitoxin.....	52	0		
Hog renin, purified (17).....	10	0		
Cascien, aqueous extract.....	150	0		
Ammonium sulfate, 0.1 sat. solution.....	110	0		

mon protein materials, such as egg albumin or diphtheria antitoxin, have proved to be inactive (table 3).

Several points of evidence can not be readily aligned with the concept that the active material was acting specifically to destroy the humoral pressor agent, however. It will be recalled that the hypertensive rat showed a refractory period after a 4 day extract treatment. This suggested that the time over which the pressure could be maintained at low levels, even with continuous therapy, could be limited. To test this point, a series of 24 rats were given daily injections of renal extract for periods of 30 to 60 days, the blood pressures being recorded daily. With but few exceptions, a full pressure return to the pre-injection level was attained within 10 to 15 days. While subsequent transitory pressure falls

were common, which often appeared cyclical, there was no tendency for the pressure to be maintained at low levels. The few exceptional animals which did show a sustained lowered pressure level failed to resume hypertensive levels when the extract injections were discontinued.

Further, the greatest pressure reduction obtainable with continuous extract-treatment was seldom much greater than that reached after the 4 day treatment. As was true for the assay, a daily dose of 0.3 R.U. was required to produce a significant pressure decline. In other words, the effects of continuous treatment did not appear to be additive.

Utilizing the same chemical procedures, extracts of various body tissues were then prepared. As shown in table 3, both hog spleen and muscle yielded active albumin in amounts comparable to that obtained from kidney. Hog blood was but weakly active. Horse serum, however, proved an exceptionally rich source of active material. As a final test, a steroid extract of hog kidney was prepared by the acetone-ethylene dichloride procedure used by Cartland and Kuizenga (18) for the preparation of adrenal cortical extract. Such an extract showed a total blood pressure lowering activity of 0.9 R.U. per 200 grams kidney, which is roughly half the activity of the protein extracts. The bulk of the activity of the steroid extract was in the fraction non-soluble in ethylene dichloride.

Since blood pressure reduction in the hypertensive rat can be obtained from albumins extracted from many body tissues, and also from a steroid fraction of the kidney, there is seemingly a strong possibility that it is a manifestation of a response to one of a limited group of foreign agents to which the body can become adapted.²

SUMMARY

The protein material contained in various hog tissues which will lower the blood pressure of the hypertensive rat has been partially purified by ammonium sulfate and acetone fractionation steps. The evidence seems to suggest that this material is contained solely in the albumin fraction. Horse serum was found to be an exceptionally rich source of the active protein.

A rat assay method for the testing of this active material has been constructed, based on the blood pressure reduction obtained in hypertensive rats after intramuscular injections of the test material over a 4 day period. It has not been found possible to maintain the blood pressure below the hypertensive level for longer than 10 to 20 days, even though extract injections were given continuously.

The reduction in blood pressure does not appear to be due to a general reaction to a foreign protein. Neither, however, can it be attributed to a single chemical entity contained solely in the kidney.

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PLASMA RETENTION, URINARY EXCRETION AND EFFECT UPON CIRCULATING TOTAL RED CELL VOLUME OF INTRAVENOUS GELATIN IN DOGS WITH DIMINISHED PLASMA VOLUME¹

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In a previous paper (1) it was shown that following a single intravenous injection equivalent in volume to 1 per cent of the body weight of a 6 to 7 per cent gelatin-saline solution in normal dogs, from 55 to 71 per cent of the gelatin had disappeared from the plasma within 6 hours and from 81 to 87 per cent in 24 hours. From 20 to 29 per cent of this gelatin was recovered in the urine during 24 hours. A marked and almost immediate decrease in the circulating total red cell volume which persisted for the experimental period of 3 days was also found.

The purpose of the experiments reported here was the study of the disappearance of gelatin from the plasma, its urinary excretion, and the effect upon the circulating total red cell volume in a condition simulating that in which it would be used as a plasma substitute.

PROCEDURE AND METHOD. The methods were those previously reported (1, 2). The dogs were anesthetized with pentobarbital sodium. A plasma volume determination was done and blood was drawn without stasis from either the jugular or femoral vein for hematocrit determination and analysis. Both hind thighs were wrapped tightly with either rubber bands approximately 1 inch wide or with rubber tubes 12 mm. in diameter. These were placed on the thighs as high as possible, tied tightly and left in place for 5 hours. It was not our purpose to produce irreversible shock but merely a reduction in the plasma volume.

One hour after removal of the tourniquets the bladder was catheterized and emptied and blood was drawn for a hematocrit determination. The animal was then given intravenously an unbuffered gelatin-saline solution containing approximately 4 per cent of alkali-treated bone collagen gelatin. The volume injected was equivalent to 2.5 per cent of the body weight. Following the injection at 6 and 24 hour intervals, plasma volume was determined and blood was drawn for analysis and hematocrit determination. The animal was again anesthetized at the 24 hour period so that this period would be comparable to the others in every respect. With dogs 1, 2 and 5 all urine was collected for 24 hours following the gelatin, the period being terminated by catheterization.

RESULTS AND DISCUSSION. In these experiments considerable vascular damage resulted from the occlusion of the blood supply. This is evident from the hemo-

¹ This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

The gelatin was furnished by Knox Gelatine Company, lot number B 78-1's.

concentration shown in table 2 at the zero hour, which was 1 hour after removal of the occlusion. In the presence of diminished plasma volume and vascular

TABLE 1
Disappearance of gelatin from the plasma

DOG	HOUR	GELATIN GIVEN	SERUM N.P.N.	SERUM PROTEIN CONC.	SERUM GELATIN CONC.	TOTAL SERUM PROTEIN	TOTAL SERUM GELATIN	GELATIN LOST FROM PLASMA	GELATIN LOST FROM PLASMA
		grams	mgm. per cent	grams per cent	grams per cent	grams	grams	grams	per cent
1	control	13.88	25.9	5.79		36.01			
	6		42.1	4.36	1.32	20.14	6.10	7.78	56.1
	24		50.3	4.01	0.44	24.94	2.74	11.14	80.3
2	control	14.70	20.1	4.82		24.39			
	6		30.0	3.23	1.44	13.82	6.16	8.54	58.1
	24		36.4	3.77	0.58	16.36	2.52	12.18	82.9
3	control	11.41	23.6	5.34		28.25			
	6		34.3	4.05	1.14	16.44	4.63	6.78	59.4
	24		37.1	3.95	0.40	20.62	2.09	9.32	81.7
4	control	14.51	34.9	6.97		51.86			
	6		79.8	5.31	1.00	28.36	5.34	9.17	63.2
5	control	16.62	26.6	7.79		85.69			
	6		23.4	6.40	0.55				
	24		26.7	6.57	0.14	72.27	1.54	15.08	90.7

TABLE 2
Changes in circulating red cell volume

DOG	WEIGHT	PLASMA VOLUME			RED CELL VOLUME			TOTAL BLOOD VOLUME			HEMATOCRIT			
		hour												
		Con- trol	6	24	Con- trol	6	24	Con- trol	6	24	Con- trol	0*	6	24
	kgm.	ml.	ml.	ml.	ml.	ml.	ml.	ml.	ml.	ml.				
1	13.5	622	462	622	505	647	545	1127	1109	1167	44.8	64.5	58.3	46.7
2	14.0	506	428	434	350	442	429	856	870	863	40.9	67.0	50.8	49.7
3	11.0	529	406	522	254	336	275	782	742	797	32.3	60.7	45.3	34.5
4	14.5	744	534	†	454	572		1198	1106		37.9	65.2	51.7	
5	17.0	1100	†	1100	680		666	1780		1766	38.2	52.3	42.5	37.6

* One hour after cessation of occlusion and just prior to gelatin injection.

† Dog died before 24 hour period.

‡ Blood volume determination lost.

damage the gelatin disappeared from the plasma at about the same rate as was the case in normal dogs. An exception is dog 5, which at the end of 24 hours had lost 90.7 per cent of the gelatin from the plasma. This animal had less vascular

damage than the others, as is seen by comparing the control and zero hour hematocrit values. The urine contained only 31.9 per cent of the gelatin which disappeared from the plasma, so the excretion was not appreciably increased. It is possible that this animal metabolized the gelatin at a faster rate than the other animals.

Urinary excretion was also determined in dogs 1 and 2. During the 24 hour period 30.5 and 37.4 per cent respectively of the gelatin lost from the plasma was recovered in the urine. These urinary values are somewhat higher than with the normal dogs, but it is believed that this difference is not significant.

In normal unanesthetized dogs a single injection of 6 to 7 per cent gelatin caused a decrease in the circulating red cell volume of from 25 to 37 per cent at the end of 6 hours (1). As will be seen in table 2, in these dogs with a diminished plasma volume, there was a considerable increase in the circulating red cell volume at 6 hours as compared with the control value. The total blood volume, calculated from the plasma volume and the hematocrit reading, remained practically constant throughout the experiments, there being an inverse relationship between the plasma volume and the red cell volume.

In this instance we are dealing with a primary deficit in plasma volume, while Hahn et al. (3) have reported an inverse relationship between plasma volume and circulating red cell volume in dogs recovering from anemia, in which there was a primary deficit in red cell volume. They state that the "total blood volume of the dog is maintained at a constant level independent of the state of anemia. As the red cell circulating volume increases, there is a corresponding drop in the plasma volume in order to maintain the total circulating blood volume constant." These observations should be verified in a variety of alterations of plasma and red cell volume before an interpretation is attempted in relation to a general physiological mechanism for the regulation of blood volume. However, the reported effect of gelatin on the circulating red cell volume is apparently of little significance in the use of this substance as a plasma substitute.

With dogs 1 through 3, the only ones with complete data, 30, 24 and 35 per cent respectively of the total serum protein deficit existing at the 6 hour period had been replenished by the 24 hour period.

SUMMARY

A decrease in plasma volume was produced in anesthetized dogs by occlusion of the circulation to the hind limbs. Infused gelatin solutions were found to leave the plasma and to be excreted in the urine at approximately the same rate as in the normal unanesthetized dog.

An inverse relationship was found between the plasma volume and the calculated red cell volume, so that the total blood volume remained practically constant. No decrease in circulating total red cell volume below the control value was found, as was the case with normal unanesthetized dogs.

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PREVIOUS DIET AND THE APPARENT UTILIZATION OF FAT IN THE ABSENCE OF THE LIVER^{1, 2}

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Work reported from this laboratory has indicated that fat-feeding has a sparing action on carbohydrate utilization during subsequent fasting (1-4). This phenomenon could be due either to increased gluconeogenesis or to accelerated fat combustion, initiated during feeding and persisting after the deprivation of food. That the latter is probably the case in the intact animal is suggested by nitrogen excretion and ketosis data (5).

The liver is commonly supposed to be the major site of the transformations involved both in gluconeogenesis and in fat combustion. The following experiments demonstrate that the carbohydrate-sparing effect of previous fat-feeding continues after abdominal evisceration, and therefore, cannot be mediated through the liver alone. It is also deduced that fat may be burned in the absence of the liver, and is, indeed, preferentially burned during early fasting following the feeding of a high fat diet for a period of time.

METHODS. Young male rats of the Sprague-Dawley strain and weighing between 60 to 75 grams were prepared for eventual evisceration by complete ligation of the vena cava above the entrance of the renal veins. The animals were then allowed to grow to adulthood on a diet of Purina fox chow fed ad libitum.

When the rats had attained a weight of 250 to 300 grams they were transferred to semiliquid diets force-fed by stomach tube (6). All animals were first fed a diet prepared by mixing equal amounts of the high fat and high carbohydrate diets described by Roberts and Samuels (3). After four days, when the rats had become accustomed to feeding by stomach tube, they were separated into two groups having similar ranges of weight. One group was fed the diet containing 85 per cent of the calories as corn oil; the other was given the diet in which 83 per cent of the calories were supplied by dextrin and 2 per cent by corn oil. The protein, lactalbumin, supplied 15 per cent of the calories in each case. Adequate vitamin and mineral supplements were included in both (3). Equi-caloric amounts of the two diets, sufficient to maintain body weight, were fed twice daily in equal volumes.

After 3 to 6 weeks on the above regimes, all animals were fasted 24 to 30 hours and eviscerated by the method of Reinecke (7). The kidneys were left intact.

The experiments performed were of two general types. In the first type, oxy-

¹ This work was part of a thesis presented by Sidney Roberts to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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gen consumption rates and blood samples were taken immediately preceding and following evisceration and at frequent intervals thereafter. In the second type, blood samples were taken as before but the animals were sacrificed five hours after evisceration, so that blood acetone body and kidney glycogen determinations could be made.

Blood samples were taken from the clipped end of the tail and, in addition, from the right ventricle of the heart at death. Simultaneous heart and tail samples checked within 5 per cent. The amount of blood obtained was either 0.05 or 0.1 cc. The tungstic acid filtrates were analyzed for "glucose" by an adaptation of the Folin-Maluro's micro-method using the Evelyn photoelectric colorimeter (3). Yeast fermentations were also done on certain of the samples to determine the extent of fermentable and of nonfermentable reducing substances present in the blood at these times. The fermentations were carried out by the method of Van Slyke and Hawkins (8). Residual reducing action of the tungstic acid blood filtrates was then determined by the micro-method previously mentioned. Non-protein-nitrogen determinations were performed on a number of the filtrates using a simple micro-modification of the Wong persulphate procedure for digestion (9), followed by direct nesslerization.

The simple manometric device designed by Shelley and Hemingway (10) was employed in the estimation of rates of oxygen consumption. The time required to consume a measured volume of oxygen, usually 25 to 50 cc., was determined. Checks of within 5 per cent were obtained consistently.

In the case of the animals sacrificed five hours after evisceration, 5 cc. samples of aortal blood were analyzed for blood acetone bodies by the method of Van Slyke and Fitz (11). The kidneys were decapsulated *in situ*, severed from arterial and venous connections after ligation, and immediately plunged into a tared tube containing hot 30 per cent potassium hydroxide. Glycogen was determined by a combination of the purification procedure of Good, Kramer and Somogyi (12) and the micro-glucose method already mentioned (3).

RESULTS. The effect of previous diet on the rate of glucose utilization and length of survival after evisceration is shown in figure 1. Feeding a diet rich in fat for 6 weeks greatly decreased the apparent rate of glucose utilization and correspondingly prolonged the survival time of rats eviscerated 24 to 30 hours after the deprivation of food.

The high carbohydrate animals showed an extremely rapid drop in blood sugar immediately after operation; all died in hypoglycemic convulsions $6\frac{1}{2}$ to $10\frac{3}{4}$ hours later (average $8\frac{1}{2}$). On the other hand, the high fat animals were able to maintain their fasting blood sugar level during this same period of time, dying only after $15\frac{3}{4}$ to 19 hours (average about 17 hrs.). Death in the latter case was not due to convulsions, but was associated with pre-mortal rigor and hyperpnea.

The yeast fermentation studies corroborated these results (tables 1 and 2). Although there was an increase in nonfermentable reducing substances in the blood with time after evisceration, the rate was essentially the same in both groups of animals (table 1). The amount of fermentable reducing substances present in the heart blood of the high carbohydrate rats at the time of death was

practically nil, while that found in the high fat group was relatively high (table 2). Evidently, the cause of death in the latter group was not a lack of glucose. The peculiar type of death noted (hyperpnea and rigor) suggests acidosis.

That the difference noted in the two groups of animals was not due to a difference in total metabolism is revealed by the oxygen consumption data. Figure 2

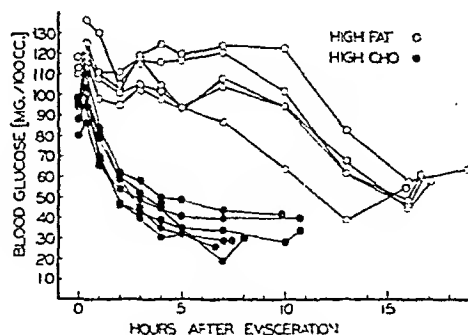


Fig. 1

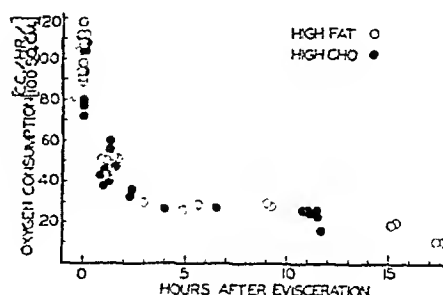


Fig. 2

Fig. 1. Blood sugar disappearance and survival time of eviscerated rats as influenced by previous diet. All animals previously force-fed the high carbohydrate diet for 6 weeks, and then fasted 24 to 30 hours prior to evisceration, died in convulsions. The high fat animals exhibited hyperventilation and progressive rigor just before death; none were convulsive.

Fig. 2. Previous diet and the oxygen consumption of eviscerated rats. All animals were fasted 24 to 30 hours after 6 weeks on the high fat and high carbohydrate diets fed by stomach tube in equi-caloric amounts. Evisceration was then carried out.

TABLE 1

Rise in non-fermentable reducing substances in the blood of rats after evisceration*

HIGH CARBOHYDRATE					HIGH FAT				
Hours after evisceration									
0	7	10	Death	Hours of survival	0	7	10	Death	Hours of survival
15	†	†	22	6½		23	20	43	15¾
11	29	†	26	7½	13	37	22		16½
9	10	†	30	8	14	35	27	43	16½
14	14	43	43	10	9	26	22	43	17
10	22		30	10¾	18	28	27	46	19
10	32		31	10½					

* Expressed in terms of milligram glucose per 100 cc. blood.

† Animal dead at this time.

clearly reveals that no significant difference existed in the oxygen consumption rates of rats fasted 24 to 30 hours and previously force-fed a high fat or high carbohydrate diet for 6 weeks. In both groups the rate shows an immediate decline after evisceration, leveling off 2 to 3 hours later. This low level of metabolism (about 25 per cent that of the intact animal) was maintained until death.

The blood and tissue analyses carried out 5 hours after evisceration in similarly-treated animals argue against the possible presence of superior concentrations of metabolites in the fat-fed group after evisceration. Thus, at this time, the blood of both groups of animals had been cleared of acetone bodies, at a time when the difference in glucose utilization was at its height. Kidney glycogen content was insignificant in all animals. The fasting levels of non-protein-nitrogen, as well as

TABLE 2

The effect of previous diet on the fall in fermentable reducing substances in the blood after evisceration*

HIGH CARBOHYDRATE					HIGH FAT				
Hours after evisceration									
0	7	10	Death	Hours of survival	0	7	10	Death	Hours of survival
73	†	†	4	6½		64	44	12	15½
83		†	3	7½	82	84	80		16½
87	9	†	2	8	101	69	68	17	16½
84	30	0	0	10	99	82	73	15	17
87	12		0	10½	102	96	96	18	19
70	8		9	10½					

* As milligram glucose per 100 cc. blood.

† Animal dead at this time.

TABLE 3

*Previous diet and the rise in blood non-protein-nitrogen after evisceration**

HIGH CARBOHYDRATE			HIGH FAT		
Initial†	Final‡	Increase	Initial†	Final‡	Increase
34	65	31	39	55	16
46	82	36	32	62	30
25	40	15	34	72	38
45	60	15	25	51	26
			35	74	29

* Expressed as milligram of nitrogen per 100 cc. blood.

† Immediately after evisceration, following a 24-30 hour fast.

‡ Five hours after evisceration.

the rate of increase during the five hours, were quite comparable in both fat-fed and carbohydrate-fed animals (table 3).

DISCUSSION. The results of this investigation show clearly that the carbohydrate-sparing effect of previous fat-feeding occurs in the absence of the liver. This phenomenon had already been demonstrated in the intact animal. Thus, animals previously force-fed a high fat diet by stomach tube showed a higher average blood glucose level after 30 hours' fasting than animals similarly fed equi-caloric amounts of a high-carbohydrate diet (1). This was associated with a slower rate of disappearance of liver and muscle glycogen (1, 2) and a reduced

sensitivity to insulin during the first 36 hours of the fast (3). The basal metabolic rates of the fat-fed rats were as high or higher than those of rats fed carbohydrate (4). That these phenomena were due to increased fat combustion in the fat-fed group was suggested by the fact that during early fasting nitrogen excretion was quite similar in the two groups, but only the high fat animals exhibited a fasting ketosis (5).

The situation in the liverless animal is probably similar to that in the intact animal. In both cases, the sparing of carbohydrate in the fat-fed group was not due to a decrease in total metabolism or to an increase in protein metabolism. Thus, in the eviscerate, rate of oxygen consumption and rate of rise of blood non-protein-nitrogen were unaffected by previous diet. Five hours after operation, kidney glycogen, blood non-protein-nitrogen, and blood acetone bodies were similar in both groups of animals. At the time of evisceration, muscle glycogen was probably quite similar in the two groups (1).

Since it has previously been shown that the fat-fed animals exhibit a fasting ketosis of about 10 mgm. acetone per 100 cc. of blood after a 24 to 30 hour fast (5), it is apparent that the eviscerate is capable of utilizing acetone bodies. However, since these rapidly disappear from the circulation after operation, they cannot be responsible for the continued slow rate of glucose utilization.

It is also evident that extra-hepatic gluconeogenesis from protein, if it occurred, was of the same order of magnitude in fat-fed and carbohydrate-fed rats. Blood non-protein-nitrogen data indicate that the differences in diet have not greatly affected this process. Gluconeogenesis in the kidney has now been demonstrated by several investigators (7, 13, 14, 15). Fat, as well as protein, may be involved here.

It seems likely, then, that the depression of glucose utilization in the fat-fed animal is due to an increased rate of fat utilization. Since the phenomenon is exhibited in the eviscerate as well as in the intact animal, it may be concluded that the liver is not essential for the utilization of fat. The work of other investigators points in the same direction. Thus, Mann (16) found that dogs previously fed large amounts of fat survived longer after hepatectomy than similar animals maintained on other diets. Drury and McMaster (17) demonstrated an R.Q. slightly above 0.7 in rabbits fasted several days prior to evisceration.

It is well established that the intact animal, while feeding, tends to burn a mixture of foodstuffs similar to that present in the diet. If the diet has been maintained long enough, this phenomenon may become selective and continue during fasting as a "preferential utilization" of the predominant constituent of the previous diet. The liver cannot be considered the sole site of this "tuning" action. Thus, it has been demonstrated above that the apparent glucose utilization rate of the eviscerate is related to the carbohydrate content of the previous diet. The situation with regard to the utilization of fat by the extra-hepatic tissues seems to be quite similar. This utilization may be direct, or it may involve some other organ such as the kidney.

SUMMARY

Adult male rats, force-fed a high fat diet for 3 to 6 weeks, and then fasted 24 to 30 hours, exhibited a much slower apparent glucose utilization rate after evisceration and survived twice as long as similar animals, previously maintained on a high carbohydrate diet. Unlike the carbohydrate-fed animals, the fat-fed group did not exhibit convulsions or a blood glucose level of practically zero at death.

Fasting oxygen consumptions, as well as the rate of decline in oxygen utilization after evisceration, were quite similar in both groups. Kidney glycogens and blood acetone bodies were insignificant in all animals five hours after evisceration. Protein metabolism, as indicated by the rate of rise of non-protein-nitrogen was unaffected by the previous diet.

It is concluded that, in animals maintained on a particular diet for a period of time, the foodstuff predominantly burned by the extra-hepatic tissues during the early stages of fasting corresponds to the major constituent of the previous diet. This applies to fat as well as to carbohydrate.

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THE INFLUENCE OF THE ARMY PACK ON POSTURAL STABILITY AND STANCE MECHANICS¹

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Modern interest in the biomechanics of posture and locomotion derives from extensive observations made during the last decades of the 19th century (1). Braune and Fischer first located the common center of gravity of the human body in the three cardinal orientation planes. From observations obtained by suspending an intact and dismembered cadaver, frozen recumbent in rigor mortis, a skeletal model was constructed to ascertain the relation of the gravity centers of the segmented parts to the adjacent joint axes. These dimensions were consigned to a living subject of similar build. By such indirect methods, the transverse vertical orientation plane passing through the center of gravity of the body could be made to coincide with the axes of rotation of the weight bearing limbs. The cardinal anteroposterior plane was assumed to divide the whole into strictly symmetrical halves. This posture called the *Normalstellung*, was used by Braune and Fischer merely as the zero position for further calculations. Subsequently this was considered to be the ideal posture of man, disregarding the limitations of the method used in the derivation of the concept and the experimental purpose for which it was originally conceived.

Braune and Fischer assumed that when once established the center of gravity of a part remains fixed. Thus they studied the mechanical characteristics of the military posture as a deviation of the body parts from the cardinal vertical orientation planes of the *Normalstellung* and determined the location of the new common center of gravity from the calculation of the degree of redistribution of the body segments. Although the major premise of the procedure is acceptable within the limits used by these investigators, it is one of questionable validity, for no two human physiques are sufficiently alike to permit a direct transference of center of gravity data from one to another; furthermore, the relations existent between the articulated parts, the position of the viscera in their respective cavities, and the pooling of the body fluids are all different enough in the vertical stance and recumbency to make the two postures incomparable.

The center of gravity of the living body in the upright stance shifts incessantly (2). The study of the oscillations of the body while standing in various postures appears to have originated with Vierordt (3) whose work was repeated and extended by Leitenstorfer's investigation of fatigue in relation to military training (4). No similar work appears in the literature. The object of this study was to observe the influence of the regulation pack of the U. S. Army

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on postural stability and alignment. Since the size, weight, and method of carrying the pack are standardized without allowing for modification in relation to variations in build and stature, the factors involved were subjected to experimental study.

METHODS. The subjects of the investigation were seven normal adult young men, five of whom were Army officers, the others were cadets in the Reserve Officers Training Corps of the University of Wisconsin. An Army sergeant

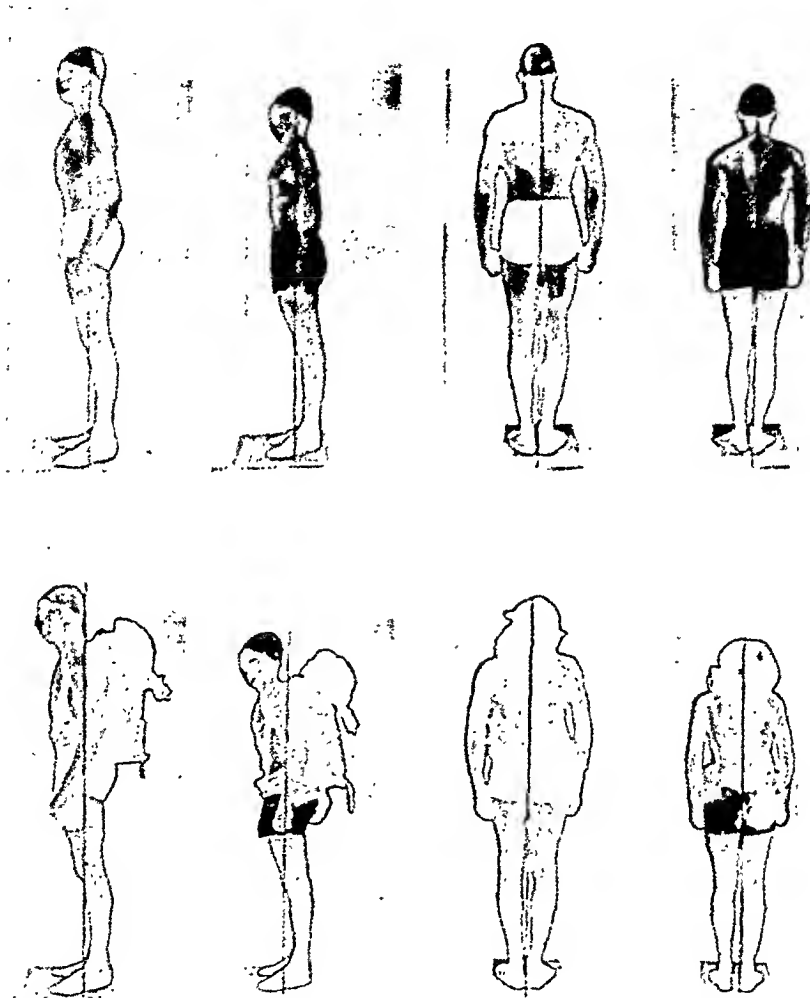


Fig. 1. Biplane negative photographs illustrating the tallest and shortest subjects in the series in the military posture and carrying the Army pack higher than regulations specify.

loaded the regulation pack and adjusted it in position. The contents of the load were then rearranged to raise or drop the center of weight and in addition the pack was carried too high on the shoulders or too low. All re-packing and adjusting were done by the sergeant. The regulation pack provided weighed 13.64 kgm. The men carried no accessory equipment (side arms, ammunition, raincoat, or blanket). The extremes in size of the men available for this study are illustrated in figure 1. The difference is 20.8 cm. in height and 31.6 kgm. in weight.

Each man was observed in the following five postures: a natural comfortable stance, the military posture, carrying the pack in regulation position, too high and too low. The methods used have been described previously (5). The subjects stood for two minutes in each posture during which biplane center of gravity observations were made at 5 second intervals. These were synchronized with sagittal and back view posture pictures. Thus 250 photographs and center of gravity observations were obtained for each subject. Experimentally determined gravity lines were introduced subsequently into the posture pictures.

A trajectory of the shifts in the center of gravity occurring in the cardinal vertical orientation planes was recorded as a moving kymogram. To gauge the manner in which the shifts in the center of gravity encroach upon the margins of static security, the common center of weight was projected vertically into the supporting base. A durable record of the weight bearing surface of the base was made by moistening the soles of the feet with potassium permanganate and having the subject stand on absorbent paper placed in a known relation to the knife edges of the center of gravity platform. The heels were separated and the feet turned out by an amount great enough to equalize the diameters of support in the anteroposterior and transverse planes as suggested by Morton (6). In the military stance, the feet were first placed in Morton's position; the subject then rose on his toes, brought the heels together and lowered them to assume the approved military posture. Since the taking of photographs made it necessary for the subject to stand in the glare of flood lights, tightly fitting dark goggles were provided so that the eyes might remain open without any increase in postural tonus due to retinal stimulation.

RESULTS AND THEIR INTERPRETATION. 1. *Natural standing.* Physiologically normal standing is not one of passive balance in the sense indicated by the coincidence of the weight line with the principal articulations in the *Normalstellung*. This has been emphasized by Steindler (7). The vertical projection of the common center of weight was placed by him at about 4 cm. in front of the ankle joint. We found it approximating the geometric center of the base in all postures both loaded and without pack, varying in respect to the axis of rotation of the ankle joint in relation to stature and the size of the feet. In the men under observation this point ranged from 6.25 to 11.73 cm. in front of the ankle joint. The relational measures of the cardinal orientation planes to various anatomical points are summarized in table 1. The toe and heel tangent data furnish demonstrable evidence for the belief that the vertical projection of the common center of weight approximates the middle of the total base, falling, as we have previously reported, slightly behind its geometric center (8). This is better expressed as an eccentricity ratio (table 2). Thus the mean vertical projection of the observed center of gravity falls behind the geometric center of the total base in natural standing by an amount equal to 7.67 per cent of one-half the anteroposterior diameter of static security. Reference to the data on coronal plane stance eccentricity shows that the vertical projection of the common center of weight falls to the left of the geometric center of the base in accord with our previous findings (8). Most of our earlier observations were

TABLE 1

The relation of the vertical projection of the center of gravity to the carriage of the head, the shoulder, the approximate area of rotation of the distal articulations of the lower extremity and to the extremes of the anteroposterior diameter of static security, based on observations made upon 6 military subjects

	NUMBER OF OBSERVA- TIONS	M.	S.D.	P.V.	V.	RANGE	
		cm.	±	±	±	cm.	cm.
Natural stance							
Head.....	149	3.71	1.96	0.12	52.63	0.00	7.01
Shoulder.....	149	4.29	1.57	0.08	36.36	0.00	7.82
Knee.....	149	-4.29	1.57	0.08	36.36	-1.96	-7.42
Ankle.....	149	-8.20	0.79	0.01	9.52	-6.65	-10.54
Toe tangent.....	150	13.94	1.55	0.08	11.11	11.33	17.42
Heel tangent.....	150	-11.63	0.56	0.03	4.80	-10.21	-12.90
Military posture							
Head.....	150	3.51	1.07	0.04	33.33	1.17	6.25
Shoulder.....	150	5.08	1.96	0.12	35.46	0.79	8.99
Knee.....	150	-4.70	1.17	0.04	25.00	-2.74	-7.04
Ankle.....	150	-8.99	1.17	0.04	13.04	-6.25	-10.54
Toe tangent.....	150	13.00	1.19	0.08	9.18	10.87	16.05
Heel tangent.....	150	-11.99	0.70	0.08	6.57	-9.68	-14.00
Pack regulation							
Head.....	150	11.33	1.17	0.04	10.34	8.99	13.69
Shoulder.....	150	9.78	1.17	0.04	12.00	5.46	14.45
Knee.....	150	-4.70	1.17	0.04	25.00	-1.96	-7.04
Ankle.....	150	-9.37	1.17	0.04	12.00	-7.04	-11.73
Toe tangent.....	150	12.93	1.19	0.08	9.23	11.28	15.54
Heel tangent.....	150	-12.62	1.02	0.05	8.05	-10.97	-14.83
Pack high							
Head.....	150	10.95	1.17	0.04	10.71	7.82	13.69
Shoulder.....	150	9.37	1.17	0.04	12.50	5.87	11.33
Knee.....	150	-4.70	1.17	0.04	25.00	-1.57	-7.04
Ankle.....	150	-9.37	1.17	0.04	12.50	-6.25	-11.73
Toe tangent.....	150	12.90	1.83	0.10	14.17	10.29	17.53
Heel tangent.....	150	-12.72	1.17	0.08	9.18	-10.13	-14.68
Pack low							
Head.....	150	11.33	1.17	0.04	10.34	7.82	13.69
Shoulder.....	150	10.16	2.34	0.12	23.08	6.25	15.24
Knee.....	150	-4.70	1.17	0.04	25.00	-2.74	-7.04
Ankle.....	150	-9.78	1.17	0.04	12.00	-7.42	-7.82
Toe tangent.....	150	13.36	1.19	0.08	8.94	9.73	14.99
Heel tangent.....	150	-13.18	0.76	0.02	5.78	-11.46	-14.63

Head: point where lobule of ear joins head. Shoulder: point most anterior. Knee: bisection opposite patella. Ankle: lateral malleolus.

made on women. The present study confirms these. There is no evidence of sex differences.

Since the weight line falls an appreciable distance from the axes of rotation of the principal supporting articulations, gravitational rotatory moments are active. These may either flex or extend contiguous segments, depending upon the relation of the application of the force to the axis of rotation of each joint. They equal in magnitude the product of the superimposed weight and the perpendicular distance of the chain of articulations from the vertical projection of the center of gravity of the body as a whole. To remain upright the rotatory components of the passively effective gravitational forces which are collapsing

TABLE 2

The eccentricity and variability of the vertical projection of the center of gravity in the unloaded stances and with the military pack

	ANTEROPosterior ECCENTRICITY				LATERAL ECCENTRICITY			
	No.	M.	S.D.	P.E.	No.	M.	S.D.	P.E.
		%	±	±		%	±	±
Six subjects								
Natural stance.....	150	-7.67	7.44	0.41	150	-7.21	3.90	0.22
Military posture.....	150	-3.16	5.97	0.33	150	-7.14	3.52	0.20
Pack regulation.....	150	-4.71	5.76	0.32	150	-6.27	4.26	0.24
Pack high.....	150	-0.91	9.84	0.54	150	-6.07	5.56	0.30
Pack low.....	150	+2.73	5.48	0.30	150	-5.87	4.90	0.27
Atypical subject								
Natural stance.....	25	-16.37	3.52	0.47	25	-8.05	1.74	0.24
Military posture.....	25	-23.20	2.38	0.32	25	-5.38	1.88	0.27
Pack regulation.....	25	-28.73	4.43	0.60	25	-5.98	1.76	0.24
Pack high.....	25	-15.52	2.46	0.33	25	-0.96	3.87	0.52
Pack low.....	25	-25.50	3.68	0.50	25	-1.46	2.56	0.34

— In back of, or to the left of the geometric center of the base of support.

+ In front of the geometric center.

in their effect must be equilibrated by muscular contractions of the extensors. An increase in postural tonus usually suffices. Phasic contractions are necessary only when equilibrium is grossly disturbed. The gravitational forces which are extending in their effect serve as muscle spacers.

The rotatory moment acting on the ankle joint is relatively large and is balanced mainly by the tonic contraction of the gastrocnemius and soleus. The forwardly unbalanced position of the leg with the foot in dorsiflexion suggests that the flexors of the toes probably contract in most subjects during natural comfortable standing, adding the accessory security offered by the digits. This enlarges and stabilizes the functional base of support. The gravity line falls in front of the axis of the knee joint. Quain suggests that the superincumbent weight of the thigh and trunk thus serves as a force which is in itself sufficient to keep the joint extended without the aid of muscular contraction (9). Little work

is being done by the quadriceps femoris in natural standing. Quain places the vertical projection of the center of gravity behind the center of rotation of the hip joint. We have no direct evidence on this point. If true, gravity passively tilts the pelvis backwards. The resultant movement is limited by tension of the hip joint ligaments. Thus the trunk is supported on the thighs in great measure without muscular effort. The muscles associated with the iliotibial band steady the condyles of the femur on the articular surface of the tibia, stabilize the pelvis on the femora and limit hip joint extension. Since the iliotibial band is attached in front of the axis of the knee joint, the gluteus maximus supports the knee indirectly in the extended position, especially when the quadriceps femoris is relaxed. In the natural stance the head, pendent upper extremities, and trunk appear to be balanced on the pelvic girdle with the musculature relaxed for the vertebral curvatures are augmented and the abdomen protrudes. The coefficients of variation in natural comfortable standing tend to be large (table 1), indicating that this position is slightly more labile than the military stance and loaded postures. This is advantageous to the circulation, providing a shifting tonus which then serves in the primary line of defense against orthostatic circulatory insufficiency and its attendant sensation of exhaustion.

Morton believes that the toes do not participate in standing and postulates that the center of weight falls midway between the heel tangent and a transverse line passing between the metatarso-phalangeal joint of the great toes. This was approached by and apparently consistently characteristic of one of our seven subjects in all postures, loaded and without pack. In our experience as close an approximation of Morton's base as this is comparatively rare. Accordingly, the observations of this subject have been excluded from the group analysis and are presented separately in table 2. Since the vertical projection of the common center of weight overhangs the axis of motion of the ankle joint of the supporting limbs by an amount which is significantly less than that evident in other subjects, this infrequently seen stance should require less muscular work to neutralize the collapsing influence of gravitational stresses. The saving in energy associated with a reduction in the size of the base by functional exclusion of the toes seems not to have been achieved at any sacrifice to stability. This is shown by comparison of the standard deviation of the eccentricity ratios in the two types of standing. Thus in one of our seven subjects the functional diameter of support postulated by Morton appears to be adequate without reliance on the accessory security offered by the toes.

2. *The military posture.* Meyer placed the gravity line in the military posture 3 cm. in front of the ankle joint according to Steindler (7). Braune and Fischer placed it at 7 cm. We found the vertical projection of the mean common-center of weight to be 8.99 cm. in front of the ankle joint for the men who use the total base in standing and 7.14 cm. in the single subject who excludes his toes. For the group as a whole this is a significant forward shift of the center of weight from that characteristic of natural standing (table 3). This represents an augmentation of rotatory stresses at the ankle joint and a small increase in moment at the knee (table 1). The weight line falls further in front of the knee joint

and therefore tends to produce overextension of the articulation; this is limited by the tension of ligaments. The relation of thigh to leg remains essentially unchanged and the superincumbent mass appears to swing forward *in toto* over the base (fig. 3). The parts at the upper end of the lever are not as forwardly imbalanced as one would expect from the augmentation of the ankle moment. The head appears to have a compensatory counterweighting action. The shoulders are retracted, swinging the arms backwards and the chest is raised, thus reducing the dorsal curvature of the vertebral column. The abdomen is flattened. The military stance suggests increased contraction on the part of hip and back extensors to balance the forwardly displaced supra-femoral mass.

The statistical analysis of the data presented in tables 2 and 3 shows that taking the group as a whole, the assumption of the military stance had no significant effect on coronal plane eccentricity but that it was associated with a significant anterior shift in the common center of weight, bringing its vertical projection closer to the geometric center of the supporting base and steadying

TABLE 3

The critical ratios of the mean eccentricity of 6 military subjects in each of the four stances: natural, the pack in regulation position, too high, and too low, compared with that of the military posture

STANCES COMPARED	ANTEROPosterior ECCENTRICITY CRITICAL RATIO	LATERAL ECCENTRICITY CRITICAL RATIO
Natural stance		
Military posture.....	5.78	0.16
Military posture		
Pack regulation.....	2.28	1.96
Pack high.....	2.39	1.98
Pack low.....	8.79	2.57

the stance. This was especially evident in the reduced variability of head poise and knee position (table 1). Thus the autonomous compensation for an augmented gravitational stress at the distal joints of the weight bearing limbs is more than adequate to meet the altered demands brought about by the new alignment. Since postural contraction is cheap and relatively indefatigable, the increased muscular effort is probably inconsequential. However, the reduction in postural sway associated with a more perfect centering of the weight above the middle of the base and the stiffening of the stance by voluntary effort might eventually prove disadvantageous because of the lessened aid thus given in combating the hydrostatic effect of gravity on the circulation. Syncope is common when the recruit is held long in a position of attention. Thus an improved centering of the weight which may be assumed to be mechanically advantageous, may not be physiologically desirable.

3. *The Army pack in regulation position, too high and too low.* A load carried on the back near the upper end of a chain of mobile segments had less effect on

postural stability than was anticipated. It was most disquieting to the stance of the taller men. However, only the tallest man in the series was distinctly

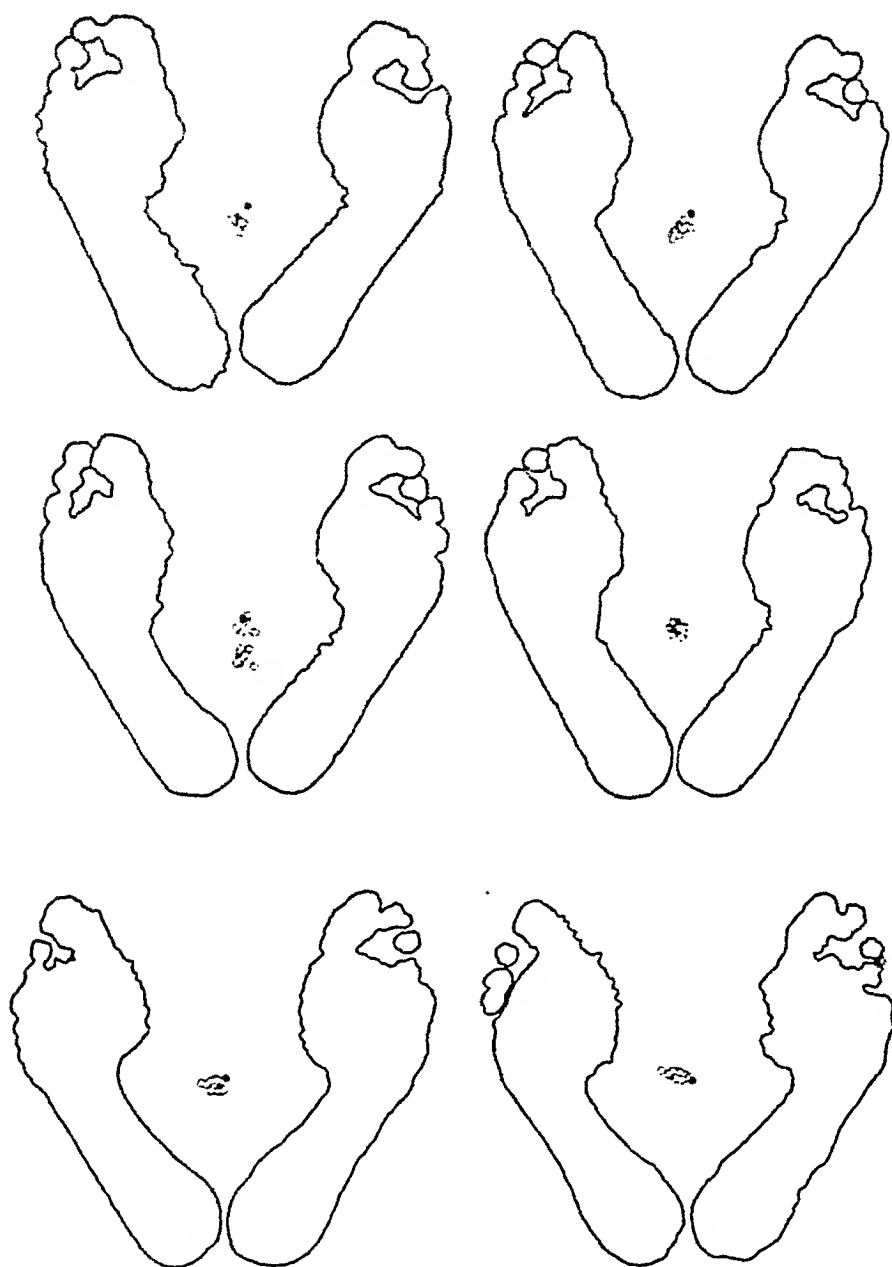


Fig. 2. Reproduction of the base of support showing the location of the vertical projection of the center of gravity at 5 sec. intervals during two minutes of standing in each of the following positions, reading from left to right: a tall subject in military posture, with the pack in regulation position, with the pack too high and with the pack too low; an average subject in the military posture and with the pack too low. The solid circle indicates the geometric center of the base.

unbalanced by the pack as illustrated in figure 2. Although he weighed more than 200 lbs., a relatively light additional load placed high on the back elevated

his already high center of gravity sufficiently to be hazardous to stability. Lowering the pack to a position below that prescribed by Army regulations had a distinctly stabilizing effect, which may not, however, be desirable for the man most in need of assistance in the prevention of gravity shock. The short man supports considerable of the pack load on the hips which is an advantage not enjoyed by the tall subjects who were forced to sustain the added weight by protraction of the shoulder girdle and an increase in counter-weighting body lean.

Figure 2 shows that the gravitational stress pattern of any one subject is characteristic and repeatable. Although a pack on the back moves the center of weight of man and load dorsally, compensatory postural adjustments are so perfectly made that no matter how the pack is carried, the subject automatically

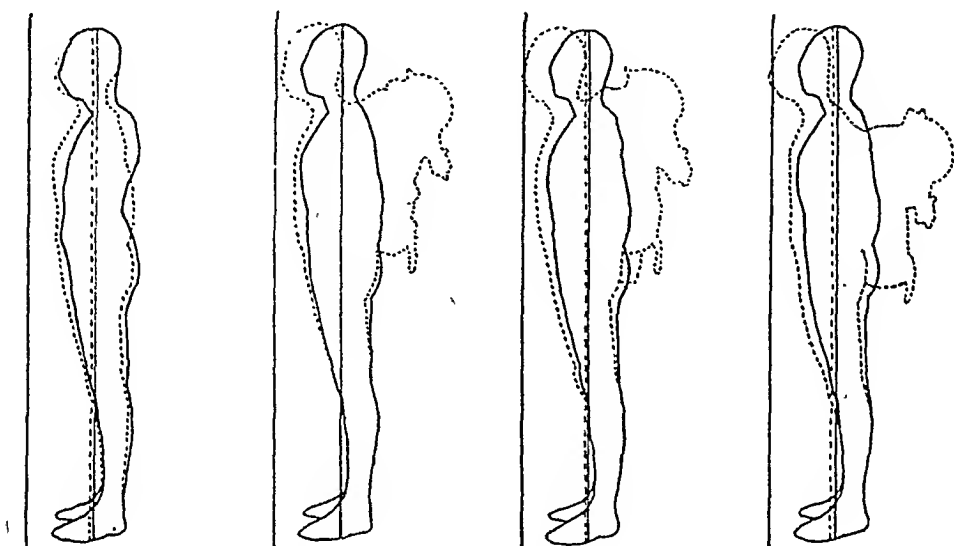


Fig. 3. Reproduction of superimposed photographs showing compensatory adjustments to stance variation and load. Reading from left to right: military posture superimposed on natural standing; pack regulation, pack high, and pack low compared with the unloaded military stance.

maintains the vertical projection of the common center of weight in much the same place as when unloaded, and always in the neighborhood of the geometric center of the base. As a whole compensation for the pack is sufficiently perfect to nullify any very great variation in postural stability save in exceptionally tall subjects. Steindler noted that there is an inherent tendency to maintain the vertical projection of the common center of weight at a constant relation to the points of support of the body. This is strikingly demonstrated in these experiments. So basic is the relation of the vertical projection of the common center of weight to the center of the supporting base that it is difficult to disrupt this principal condition of equilibrium.

The major compensatory adjustment to the backwardly displacing influence of the pack appears to be an increase in the flexion of the leg upon the foot,

further augmenting its normal forwardly unbalanced position. With the pack in high or in regulation position there is no significant displacement of the center of gravity as a whole giving rise to unnatural stresses differing from those evident in the military posture of which the loaded stance is a modification. The head and shoulders swing forward in an effort to equalize the load resting on the back as body lean is augmented (fig. 3). Since the anteroposterior eccentricity of the center of weight has not altered significantly, these postural adjustments balance the load cancelling its effects on the center of weight. There is no evidence of rotation of the pelvis on the heads of the femora in the adjustment of the load. If this were occurring, concomitant flexion of the knees would be apparent for each segment of the chain of articulated parts which sustain the body in the upright posture compensates automatically for disturbances in the alignment of its neighbors. Except for the muscular contraction necessary to carry the pack and an increase in stress at the ankle, there is no indication in our data of an additional demand on knee, hip or back extensors beyond that called forth by the unloaded military posture. When the pack was carried low, the center of gravity shifted significantly forward so that its mean position for the men who use the toes in standing fell in front of the middle of the base, increasing the strain on the foot (fig. 2). Thus overcompensation ensued when the pack was borne low, perhaps in part because fewer segments participate in the balancing readjustments, reducing the sensitivity of the system. Or more important, because the low placed load by augmenting the backward displacement of the center of gravity of man and pack increases the magnitude of the equilibrating forward body lean. The stance assumed when the pack was lower than regulations require was less asymmetric in the coronal plane than any other posture taken by this group of men and was the least variable. Except for the high pack carried by the tallest man, all loaded postures were steadier than those without pack. It is known that distraction reduces postural sway. Preoccupation with the problem of balancing the load appears to suppress the corticofugal impulses which inhibit postural tonus.

SUMMARY AND CONCLUSIONS

The influence of the regulation Army pack on the symmetry and stability of the stance and upon the magnitude of the gravitational stresses acting on the joints of the supporting limbs was investigated on five Army officers and two cadets of differing build, ranging widely in weight and stature. The evidence from 1750 center of gravity observations synchronized with stance photographs substantiates the following conclusions:

1. Postural sway is inseparable from the normal stance of young adult men in military training.
2. The military stance reduces postural sway.
3. Postural sway is suppressed still further by the carrying of a pack.
4. A too high placement of the pack tends to unstabilize the stance of tall men.
5. Strain on the foot is greater when the pack is carried lower than regulations require since the center of gravity swings to a position in front of the center of the base.

6. All stances, natural and military, loaded and without pack, are slightly eccentric, the vertical projection of the center of weight falling to the left of the geometric center of the total base and with the exception of the low packed stance slightly behind it.

7. To counterbalance the pack, the body leans forward *in toto* over the ankle joints without significant realignment of other segments.

8. The compensatory adjustments to a load carried on the back are sufficiently good to enable the vertical projection of the center of gravity of the body as a whole to remain within ± 7 per cent of the geometric center of the base irrespective of how the regulation Army pack is carried.

9. The small man exhibits no disadvantages when carrying a pack which cannot be met adequately by automatic compensatory adjustments.

10. Since the stance assumed with the pack in regulation position most nearly approximates military and natural standing in the mean position of the vertical projection of the center of weight as related to the margins of static security, it probably represents the best autonomous compensation for the load.

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THE INTERACTION OF MYELINATED FIBERS IN MAMMALIAN NERVE TRUNKS

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Two years ago Dr. E. Th. von Brücke made the interesting suggestion that the periods of supernormal and subnormal excitability which follow the activity of nerve fibers might be due to chemical, rather than to electrical events. Doctor Brücke's untimely death prevented our carrying out experiments we had planned in order to clear this problem. The present paper deals with some of those experiments. The main interest of the study was shifted to the problem of the interaction among the several axons of a nerve trunk. Other observations, therefore, have been added, concerned with this interaction.

METHOD. Cats were used, anesthetized with dial (Ciba, 0.7 cc. per kgm. intraperitoneally) or with nembutal (Abbott, 0.6 cc. per kgm. intraperitoneally), or else decerebrated under ether, with a sufficient lapse of time allowed for elimination of the anesthetic. The nerve trunk studied was the peroneal, either in situ and circulated or else excised and placed in a moist air chamber.

The diagram in figure 1 indicates the position of the several stimulating and recording electrodes. These electrodes were chlorided silver needles. When the nerves were in situ the sciatic was cut as far centrally as possible, above an artery which supplies the nerve in the region of the hip. The peroneal nerve was then separated from the popliteal and hamstring trunks down to the level of the small artery which regularly enters the nerve below the trochanter. This part of the peroneal was enclosed in a glass tube (Sherrington electrodes *T 1* and *2* in the diagram). Although the longitudinal vessels of the sciatic were carefully preserved along the peroneal, this part of the nerve usually showed signs of deficient circulation, as will be explained later. The test electrodes *T 3* and *4* were hooks, shielded by rubber, and were applied to the peroneal and popliteal trunk (below the hamstrings) at the upper part of the thigh, between two of the side arteries which join the nerve; this part of the nerve always had normal circulation. The recording electrodes *R* were of the Sherrington type. They were applied to the superficial branch of the peroneal. The circulation of this branch was always excellent. Finally, the conditioning electrodes *C* were applied to all the other branches of the peroneal, after peripheral crushing or cutting.

The nerve action potentials were photographed from a cathode-ray oscillograph. Two 5-stage amplifiers were used. One was capacity-coupled, with a time constant of 0.5 or 0.05 sec. It was employed exclusively for measuring spike amplitude. The other was direct-coupled, and was used for recording both spike and afterpotentials. The frequency characteristic of this amplifier is as follows: up to 2,000 cycles the output is linear; at 9,000 cycles the output is 50

percent of that for low frequencies. The drift of the amplifier is less than $5\ \mu\text{v}$ per min. With input electrodes both on live nerve the drift was usually less than $10\ \mu\text{v}$ per min. Both these amplifiers were designed and built by Albert M. Grass.

The input to the amplifiers was on push-pull. The animal, or the excised nerve, was grounded either through a diffuse indifferent lead or through one of the stimulating electrodes.

Two stimulators were used, one for repetitive, the other for 2-shock excitation. Each permitted the accurate timing of the pulses in two independent outputs. In the repetitive stimulator, constructed by A. M. Grass, condenser discharges through a thyatron overload a critical d.c. amplifier, with positive feed-back. The pulses become thus quite rectangular. The duration of these pulses is determined by the capacity discharging through the thyatron. The thyatrons for the two pulses are overbiased. They are tripped by a.c. from an oscillator. This a.c. is not delivered directly, however, but is passed through two capacity-resistance bridges which change its phase independently for each thyatron. Pairs of shocks can thus be obtained at any given frequency, with the two pulses in the pair separated by a continuously variable time interval.



Fig. 1. Diagram of the circulated peroneal nerve, cut centrally (left) and peripherally (right). The electrodes were used as follows: *T*, for testing; *R*, for recording; *C*, for conditioning.

The two shock stimulator consisted of two thyatrons controlling condenser discharges. One of these thyatrons was tripped directly by a condenser pulse, the other only after a delay imposed by resistance and a shunt capacity.

The duration of the rectangular pulses from the repetitive stimulator was usually 0.1 msec. The time constant of the condenser discharges in the 2-shock stimulator was about 0.08 msec. The pulses were often not delivered directly to the nerves, but passed through a transformer in order to render them diphasic and to avoid the ground connections of the stimulators.

The injections of drugs were made into the aorta, just below the inferior mesenteric artery. Since the leg was routinely fixed by clamping two drills inserted into the femur, movements of the animal did not change the contacts of the stimulating and recording electrodes.

RESULTS. A. *The changes of electrical excitability of axons determined by the passage of nerve impulses in adjacent fibers.* These changes were explored by stimulating first the fibers in one of the branches of the nerve (conditioning stimuli, electrodes *C* in fig. 1) and then testing the excitability of the fibers in the recording branch (electrodes *R*, fig. 1) by means of submaximal stimuli applied

after a variable time interval to the whole trunk more centrally (test electrodes T_1 to 4). The indicator of the changes of excitability was the amplitude of the conditioned spike potentials. If these increased a larger number of fibers was activated by the test shocks. Since the shocks were of constant characteristics an increase of response thus indicated that the test stimuli had become liminal for fibers for which they had been subliminal without conditioning, hence, that the nerve was more excitable. Conversely, a decreased response indicated decreased excitability.

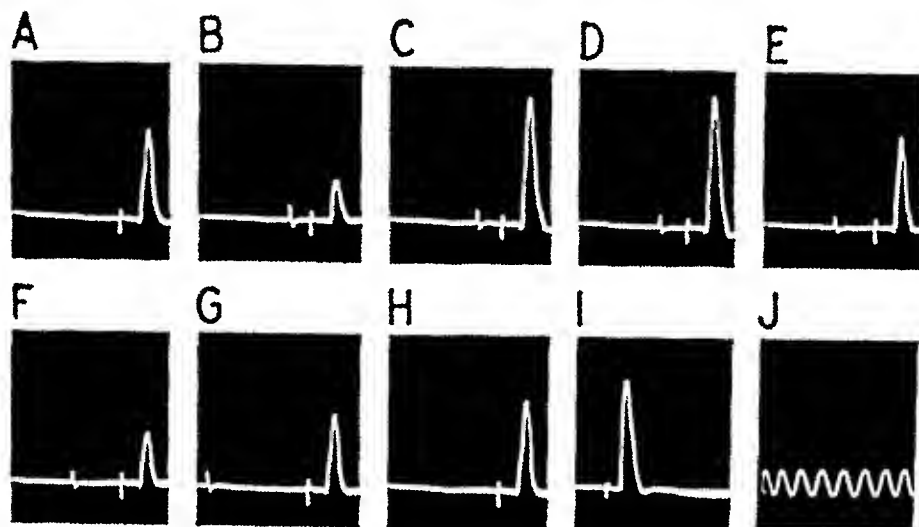


Fig. 2. Changes of excitability of axons determined by nerve impulses in adjacent axons. Submaximal constant test shocks applied continuously to electrodes T_1 and T_2 (cathode; see fig. 1) with a frequency of 20 per sec. Conditioning maximal shocks applied intermittently to electrode C (fig. 1) with the same frequency and variable timing with respect to the test shocks. Monophasic records from electrodes R . Each picture corresponds to 6 to 8 responses.

A. Control response without conditioning.

B to G. Responses with conditioning at increasing intervals, as shown by the position of the conditioning stimulus artifact.

H. Unconditioned control response at end of series.

I. Response recorded from the test electrodes (T_1 , T_2) to stimulation by the conditioning shocks.

J. 1,000 cycles.

The usual procedure was to adjust the intensity of the unconditioned test shocks so that they yielded a response with an amplitude of 20 to 40 per cent of the maximal A spike. Photographs were then taken of these responses and of those which obtained when conditioning stimuli were delivered at various times before the test shocks. Conditioned and unconditioned responses were regularly alternated. The interval between the stimuli was first progressively increased and then decreased, or vice versa. Since the phase shifters of the repetitive stimulator had a potentiometric control of resistance, a continuous variation of the shock interval could be obtained by merely turning a knob. The intervals which led to significant changes of excitability could thus be readily identified. In figure 2 are reproduced typical records of conditioned and unconditioned re-

sponses with test and conditioning stimuli at a frequency of 20 per sec. The film was exposed about 0.3 sec. Each picture corresponds therefore to about 7 superimposed responses. The accuracy of this superposition bears witness to the regularity of the responses. Such regularity was the rule.

After a series of observations had been photographed (fig. 2, A to H), the responses to the conditioning stimuli were recorded with the same sweep velocity from the previous test cathode to the test electrode *T 1* (fig. 2 I). This record showed the precise time of arrival of the conditioning impulses to the region of the nerve where the changes of excitability were tested. All the records were later measured by projection through a photographic enlarger and curves such

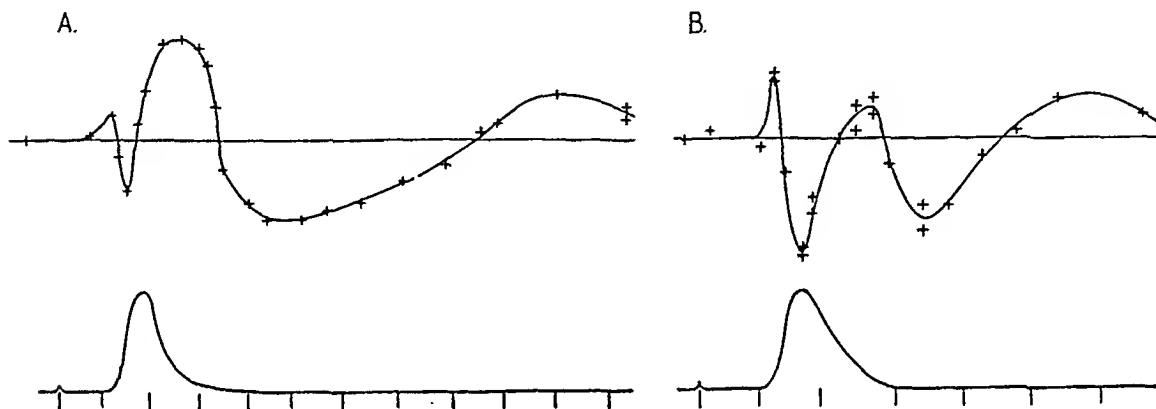


Fig. 3. Changes of excitability of axons during and after the passage of nerve impulses in adjacent fibers. Submaximal test shocks applied continuously at the rate of 40 per sec. Conditioning shocks applied with the same frequency, but timed at various intervals before or after the corresponding test shocks. Ordinates: amplitude of the responses to the test shocks in conventional units; the horizontal line indicates the amplitude of the unconditioned responses. Abseissae: time; the scale corresponds to 0.5 msec. intervals. The abseissae of the different points indicate the time of application of the corresponding test shocks with respect to the arrival of the conditioning nerve volleys to the test cathode. The lower tracing shows the spike potential of a conditioning volley recorded from the test electrodes.

The test cathodes were 6 and 20 mm. away from the crushed central end of the nerves in A and B, respectively.

as those in figures 3 and 4 were drawn, correlating the changes of excitability with the arrival of the conditioning impulses at the test cathode.

As will be described below, the results varied with the experimental conditions. This variability, however, was not so great as to eliminate the recognition of certain regular features illustrated in figure 3. An increase of excitability precedes the arrival of the nerve impulses at the test cathode. The rising phase of the conditioning spike potential is attended by a decrease and the falling phase by an increased excitability of adjacent axons, so that the curve inflects suddenly at about the crest of the spike potential. These changes are followed by a later fall and a still more delayed increase of excitability, which occur after the spike has subsided, in the course of the after-potential sequence.

A significant factor which modified the curves of figure 3 was the position of

the test cathode on the nerve—i.e., the region of the nerve where the changes of excitability were explored. In general, if that region was near (0.5 to 1 cm.) the damaged central end of the nerve the crests of increased excitability were prominent and the troughs of decreased excitability were slight (fig. 3 A) or, exceptionally, absent. Conversely, when the region tested was far (2 to 5 cm.) from the central crushed end, and hence more normal, the crests were usually slight, or exceptionally absent, while the troughs were usually prominent (fig. 3 B).

The frequency of stimulation, that is, the number of tests made per unit time, influenced only slightly and irregularly the curves of figure 3, within the range studied (from 1 per 3 sec. to 60 per sec.). In general, however, the first rise of

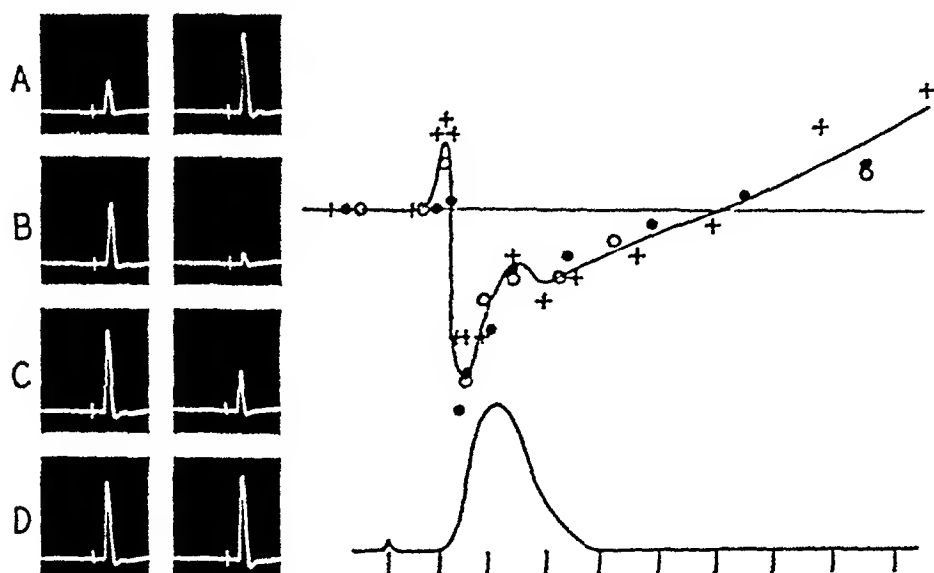


Fig. 4. The absence of effects of cathodal or anodal polarization of the tested region of the nerve on the changes of excitability caused by conditioning volleys. The curve in the graph corresponds to observations similar to those described in figure 3. The crosses measure the changes of excitability before the application of direct current. A shows the increase of responses when cathodal polarization was started. The circles in the graph correspond to this condition. At B the d.c. was discontinued; the drop of response shows that the nerve was effectively polarized throughout the period. At C anodal polarization was started after readjustment of the intensity of the test shocks. The dots in the graph correspond to this condition. At D the d.c. was broken; the rise of response indicates effective polarization during the period of observation.

excitability, which precedes the arrival of the conditioning nerve impulse at the tested region, was more prominent with frequencies of 30 to 60 per sec. than it was with slower rates of testing.

The results were similar in the nerves in anesthetized cats to those obtained in the decerebrate animals. They were only slightly and irregularly different when circulated nerves in situ were compared with excised trunks.

The polarity of the test shocks had no significant influence on the results. Thus, similar changes of excitability were observed when the cathode of the test stimuli was at electrode *T 3* of the diagram in figure 1 while the anode was at *T 2* or *T 4*.

The influence of cathodal and anodal polarization of the region of the nerve tested was studied as follows. First, measurements such as those illustrated in figure 2 were made with a given position of the test electrodes (e.g., cathode *T* 3, anode *T* 4, fig. 1). Then direct current (d.c.) was applied so that the test cathode was either the cathode (e.g. cathode *T* 3, anode *T* 2) or the anode (anode *T* 3, cathode *T* 2). The current was slightly above or below threshold, so that the test pulses now yielded larger or smaller responses, depending on whether cathodal or anodal polarization was applied. The test stimuli were then adjusted to give responses approximately equal to those used in the first series and a new set of observations of unconditioned and conditioned responses was made while the d.c. was flowing continuously. Then the current was discontinued, whereupon the amplitude of the test responses changed in the reverse direction to that which had occurred when the d.c. was first applied, thus showing that the polarization had been effective throughout the period of observation.

In figure 4 is illustrated a typical experiment. The crosses in the graph represent the control observations. Cathodal polarization was then started as shown by the change of responses in A. The observations corresponding to the circles were then made and the d.c. was discontinued (B). The d.c. was then reapplied with the polarity reversed (C). The pictures corresponding to the dots in the graph were taken and the d.c. was again discontinued (D). It is clear that neither cathodal nor anodal polarization with currents of about threshold strength cause any significant change of the curve depicting the variation of excitability. With strong currents (2 to 4 times rheobase) the curves were flat, that is, the changes of excitability were minimized, especially when the polarization was anodal.

B. *Interaction of nerve fibers after injections of veratrine.* All these experiments were carried out in anesthetized cats, because it was found that the decerebrate animals could not survive injections of even small doses (0.5 to 1 mgm. per kgm.) of veratrine. Death was due to circulatory failure, for it was not prevented by artificial respiration. With doses of veratrine smaller than the lethal amount the motor activity of the cats was extreme and the effects described below did not appear.

In a series of observations submaximal shocks were applied at frequencies of 1 to 100 per sec. to two of the test electrodes *T* (fig. 1). Single maximal conditioning shocks were then delivered in the course of the test train to electrodes *C*—i.e., to nerve fibers which did not contribute to the record (electrodes *R*). When this procedure was followed without veratrine only a few (0 to 3) test responses were modified, as the curves in figures 3 and 4 would suggest.

After injections of veratrine (0.5 to 4 mgm. per kgm., intra-arterially) the results were different. As shown by Gasser (1938), veratrine promotes the appearance of recruitment of nerve fibers during repetitive stimulation—i.e., each successive shock in a repetitive train tends to elicit a greater response than the preceding one. The intensity of the shocks may be adjusted, however, for any frequency, so that after a variable period (e.g., from 1 sec. to 3 min.), which depends on the dose of veratrine and on the frequency of stimulation, recruitment

stops, or becomes very gradual, while the test responses are well submaximal. If a single conditioning shock was delivered after this equilibrium of the test responses had been attained, after a brief delay (0.05 to 0.5 sec.) the responses increased progressively and markedly, so that they could grow from barely liminal to practically maximal. Figure 5 illustrates a typical observation. With relatively small doses of veratrine and relatively low frequencies of stimulation (see below) this increase was reversible, i.e., within a few seconds or minutes the responses gradually subsided to their original level (fig. 5A). If the dose of veratrine and the frequency of the test shocks were relatively high the increment of the responses did not subside as long (up to 5 min.) as the test shocks were not interrupted. A brief interruption (0.5 to 2 sec.) led back to the initial conditions.

The term "relative dose" of veratrine was used above because, as pointed out by Acheson and Rosenblueth (1941), the amounts of the drug which elicit any of the several effects it exerts vary considerably from cat to cat. For the appearance of the present phenomenon relatively large doses had to be employed. After injections sufficient to elicit marked repetition of the nerve impulses in response to single shocks and a marked increase of the negative afterpotential, the phenomenon could be entirely absent. The usual procedure was to inject an amount sufficient to produce a brief period (1 to 10 min.) of total block of the nerves. After recovery the effect could be studied for hours.

That the phenomenon was not correlated with the presence of a large negative afterpotential was shown by its absence in the conditions cited above. Furthermore, in several experiments in which the observations were carried out for 6 to 9 hours after a single sufficient injection of veratrine, the negative afterpotential decreased with time, so that it was only slightly greater than normal, while the increase of excitability after single conditioning of adjacent fibers was still prominent.

Two correlations were found between this phenomenon and other effects of veratrine on nerve. The first was positive: the phenomenon never appeared unless there was good recruitment; and conversely, if there was marked recruitment the increase with conditioning was present. The other was negative: whenever there was a marked drop of spike potential amplitude with repetitive stimulation (see Acheson and Rosenblueth, 1941), the phenomenon was minimal or absent. Indeed, the few (4) experiments performed under nembutal anesthesia were quite unsatisfactory—under nembutal the decrease of spike amplitude was found much more striking than under dial.

For a given degree of veratrinization recruitment is more prominent with high than with low rates of stimulation (see Gasser, 1938), within a certain range which depends on the dose of drug. The terms relatively low or high frequency were used to indicate rates which allowed a slight or a prominent recruitment, respectively.

The phenomenon of figure 5 did not take place unless the injected veratrine had reached the region of the nerve tested by the continuous submaximal shocks. Thus, in some experiments the increase after conditioning was striking when the test cathode was *T 3* or *T 4* (fig. 1), but it did not occur if the test cathode was

T 2. In those cases, the records from stimulation at *T* 2 showed the signs of veratrinization of the recording region—e.g., there was a large negative after-potential—but stimulation of *T* 2 did not lead to any appreciable recruitment. Obviously the blood supply had been impaired at *T* 2.

The results of repeating the conditioning stimuli during a continuous train of test shocks were as follows. In a series of experiments repeated conditioning volleys (several slow shocks, or brief trains of pulses at about 6 per sec.) were applied without accurate timing with respect to the test stimuli. Several rapid conditioning shocks did not cause in general any longer lasting or greater increment of the test responses than did a single shock. When a first effective conditioning stimulus was followed 5 to 30 sec. later by one or more additional shocks, the latter had usually no increasing effects, even if the increment occasioned by the first conditioning shock had largely or entirely subsided. Full reduplication of the first effect was obtained only if further conditioning was applied at least

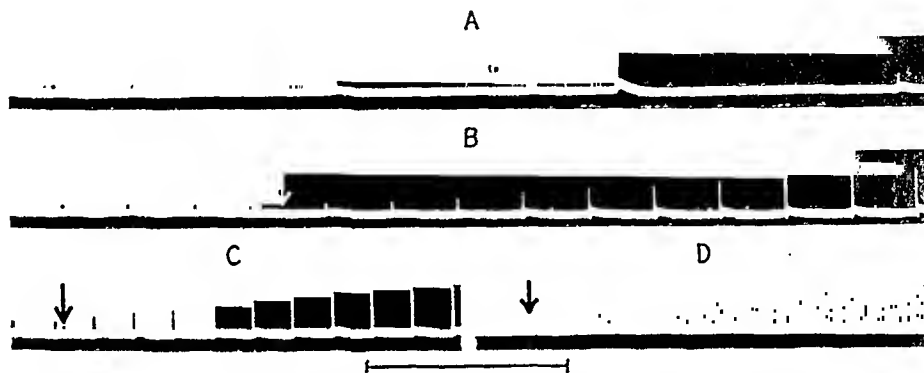


Fig. 5. Increase of submaximal test responses when conditioned by single stimulation of adjacent axons, after veratrine. Records and stimuli as in figure 2. Veratrine (1 mgm. per kgm.) injected 3 hours previously. The test stimuli were applied, with the frequency corresponding to each record, for several minutes, until equilibrium was attained. The stimulus artifacts were small and do not appear on the records. At arrows a single maximal shock was delivered to the conditioning electrodes, A to D, increasing frequencies of test stimulation. Time calibration: 1 sec.

about 1 minute later. In favorable preparations successive shocks at a relatively slow rate (about 2 to 4 per min.) could prolong, however, the increasing effects obtained from a single conditioning pulse.

In another series of experiments the conditioning stimuli were applied at the same frequency as that of the test shocks and the time between each two pulses was varied. Within a certain range of shock intervals the conditioning stimuli could be applied without any increase of the test responses as long as the conditioning train was sustained. Thus, if a series of test stimuli was initiated, and after equilibrium conditioning shocks were started preceeding each test stimulus by about 2.5 msec., the test responses were not modified. This stable situation could be maintained for several minutes, if none of the conditions were varied. If the conditioning train was stopped, however, the test responses promptly increased (fig. 6A), reversibly or irreversibly, as explained before for single shock

conditioning (p. 662). If, without interrupting the conditioning train, the interval between the two stimuli—conditioning and test—was made either too brief (less than 2 msec.) or too long (more than 4 msec.), then again the test responses promptly increased (fig. 6B).

If the time intervals mentioned above are referred to the time of arrival of the conditioning nerve volleys to the tested region, then the results may be restated as follows. As long as the test stimuli were applied during the period corresponding to the falling phase of the conditioning spikes or shortly thereafter, the increment of excitability elicited by the conditioning volleys was held in abeyance. As soon as any test shocks were applied outside this period—either by interrupting the conditioning stimuli or by displacing them in time—the increment appeared.

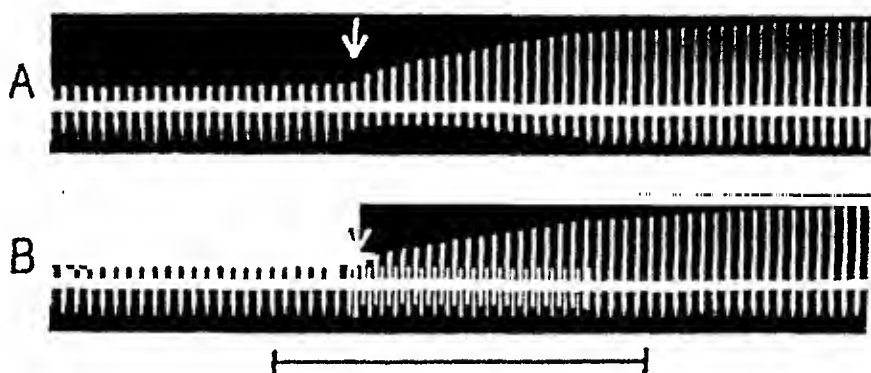


Fig. 6. The phenomenon of figure 5, but with repetitive conditioning stimuli of the same frequency as that of the test stimuli.

In A the test shocks were first started and delivered until equilibrium. Conditioning shocks preceding the test stimuli by 2.5 msec. were then applied. The responses were not modified. The record begins at this stage and shows the increase of responses which occurred when, at the arrow, the conditioning shocks were interrupted.

In B the initial steps were as in A. The record begins with both stimuli separated by an interval of 2.5 msec., as before. At the arrow, without stopping the conditioning train the timing between the pairs of stimuli was changed so that the conditioning preceded the test shock by 15 msec.

The results suggested, therefore, that after veratrine the conditioning volleys have an enhancing influence upon the electrical excitability of neighboring axons, but that they also exert a depressing influence during a period which corresponds approximately to the stage of decline of the spike potential. This suggestion was tested by means of timed 2-shock observations and was confirmed.

C. Acetylcholine, Adrenaline and K Cl. The effects of intra-arterial injections of these substances were studied in the course of trains of submaximal test responses obtained from stimulation of electrodes *T 3* and *T 4* (fig. 1). Adrenaline (up to 50 γ) had no action on the electrical excitability of the nerves, whether injected before or after veratrine. Acetylcholine (up to 50 γ) had likewise no effects, even when it was tried after an injection of prostigmin (0.5 mgm. intravenously).

Injections of K Cl, on the other hand, resulted in transient (0.5 to 5 min.) increases of the test responses. The effects were sensitized by veratrine. Thus, in a typical observation 10 mgm. of K Cl had no results while 25 mgm. caused a moderate, brief (about 30 sec.) increase of the responses. After veratrine (0.5 mgm. per kgm.) a marked, prolonged increment took place with 5 mgm. of K Cl, and even 2.5 mgm. caused clear effects. The records were quite similar to those in figure 5.

DISCUSSION. A. The only step of the experimental procedure which needs a comment is the use of the spike amplitude as an index of changes of electrical excitability. The statistical distribution of the thresholds of A fibers in mammalian nerves is skewed. When the spike amplitude is plotted against the intensity of shock an asymmetric S shaped curve is obtained (see Rosenblueth and Rioch, 1933). Within the range of spike amplitudes used in these observations, however, this curve is fairly linear. It may be concluded, therefore, that the changes of spike amplitude within that range are approximately proportional to the changes of threshold. Only few measurements were made of the actual thresholds. It was found that doubling or halving of the spike amplitude denoted a change of about ± 10 percent of the threshold of a given number of nerve fibers.

B. Experiments similar to those illustrated in figures 2 and 3 have been made by Blair and Erlanger (1940) and by Renshaw and Therman (1941). These authors studied only the changes of excitability which took place in fibers during the time of development of the spike potential in adjacent fibers. They report that in a normal region of the nerve (away from a damaged part) the excitability decreases during the period of development of the spike, while in regions close to a damaged part the excitability increases at that time.

Blair and Erlanger suggest that the increase of excitability is due to the stimulating action of the spike and the decrease is caused by shunting of the test stimuli due to the decrease of electrical impedance of the active fibers which attends the passage of a nerve impulse (Cole and Curtis, 1939). Both these opposing factors would be at play in any part of the nerves tested, but one or the other would preponderate at any region, depending upon the local conditions. The regions close to an injury would show the increase rather than the decrease of excitability because they would be constantly and effectively in a condition equivalent to a cathodal polarization, as a consequence of the demarcation potential.

The present data agree with those cited in that the results differ in regions near an injury from those found in parts distant from the injury. The observations of Blair and Erlanger are also confirmed, that the changes are exclusively determined by the position of the test cathode and are independent of the polarity of the test stimuli (see p. 660). The present data indicate, however, that the changes of excitability of inactive fibers determined by the passage of a nerve impulse in neighboring elements are more complex than implied by the simplified description of the previous studies, and that they may not be directly or simply correlated with the development of the spike potential.

The explanatory suggestions of Blair and Erlanger are not supported by the data. It is not likely that the shunting effect of active elements is significant in the experiments, as follows. First, in no instance in these or previous observations was there a decrease of excitability with the time course described by Cole and Curtis (1939) for the change of impedance. Second, this shunting effect can only be minimal when, as was often done here (see Method), the test electrodes included a large proportion of shunting unconditioned elements (the popliteal nerve). Since there was a low resistance shunt at all times it is unlikely that a slight further shunt should have any significant influence.

The statement that the spike potential may excite neighboring fibers requires expansion and clarification. The effective electrical field might be the localized one corresponding to the development of the spike potential at the region of the conditioning fibers in the immediate vicinity of the test cathode. One would then expect a change of excitability of the surrounding elements which would have the time course of the spike. This expectation is not supported by the data, since there may be a reversal of the excitability change in the course of the spike (fig. 3). On the other hand the effective field could be modified by the travel of the conditioning impulses to and beyond the tested region, and hence the field would be variable not only in time, but also in spatial distribution. This field will exhibit two reversals. Just before the propagated wave reaches an abstracted point under consideration, that segment will be relatively electropositive with respect to the then active stretch. The point will next be electronegative with respect to the rest of the nerve, when the wave has reached it. It will later again be relatively positive with respect to more distant regions, when the wave has travelled further. The experimental results support the view that the electrical fields set up during the activity of a given point in a given fiber influence the excitability at the corresponding points of adjacent elements, since the curves in figure 3 show inflections precisely at the time when the field varies. A corollary of this argument is that relative electropositivity of the conditioning fibers at the segment tested enhances the excitability of the adjacent elements, while electronegativity depresses this excitability. Since the interaction between fibers is independent of the polarity of the test shocks (i.e., ascending or descending test pulses), it may be inferred that the longitudinal component of the electrical fields under consideration is negligible and that the transverse or perpendicular components are the only effective influence.

The suggestion of Blair and Erlanger (1940), that the difference between parts of the nerve near to and those far from an injury is due to a cathodal polarization determined by the demarcation potential, is not supported by the observations illustrated in figure 4. Neither cathodal nor anodal polarization of a region far from an injury leads to any important change of the results. If the demarcation potential is dismissed as a significant factor the peculiar effects encountered near an injured part may be attributed to the relatively small amplitude of the spike potential and to the atypical distribution of the corresponding fields.

C. The discussion has dealt so far only with the early changes of excitability, which occur during the development of the conditioning spike potential. The

curves in figures 3 and 4, however, show that there are marked later changes, first a depression and then an increase of excitability, which long outlast the spike process. This sequence might suggest that the decrease could correspond to the negative, and the increase to the positive afterpotential, respectively. This suggestion is not favored by the following considerations.

The amplitude of the afterpotentials in normal, unpoisoned nerve is very slight, compared to that of the spike potential. The electrical influence on neighboring fibers should be correspondingly negligible. Yet the late changes of excitability may be quite as striking as those which occur during the development of the spike. Furthermore, after veratrinization the late changes of excitability are not correlated with the prominent negative afterpotential (see p. 662).

If a direct electrical influence is dismissed, an alternative explanation for the late changes of excitability is the diffusion of chemical substances from or into the active fibers, and the consequent alteration of the surroundings of the same and of other inactive fibers. This alternative hypothesis does not preclude the possibility that the chemical changes in question may alter the electrical equilibrium of the conditioned elements.

In 1886 Wedensky reported that a single maximal stimulus to a pair of electrodes on a motor nerve could result in a tetanic muscular response if subliminal repetitive stimuli were simultaneously applied to another pair of electrodes on the nerve. He called this phenomenon "the tetanized twitch". Samojloff (1930) attributed the tetanic response to a repetitive discharge at the neuromuscular junction. Later Kisseleff (1934) showed, in agreement with Wedensky's original interpretation, that the source of repetition was the test stimuli, subliminal unless conditioned. Kisseleff attributed the phenomenon to super-normal excitability attending the after-potential of the conditioning volley.

Excepting for the inclusion of the tested fibers in the conditioning volley, Wedensky's phenomenon in unpoisoned frog's nerves is similar to that illustrated in figures 5 and 6 after veratrinization. The belated, slow, reversible increment of the test responses, elicited by a nerve volley over the conditioning fibers in figure 5, is readily accounted for by the assumption that this nerve volley releases a chemical agent which diffuses around the tested elements and modifies their excitability.

The fact that a second conditioning stimulus, applied about 30 sec. after an initial effective one, had only minor or no enhancing effects, even when those from the first stimulus had subsided (p. 663), is opposed to the concept of an electrical interaction, since the electrical response to the second shock is similar to that to the first in those conditions. According to the chemical interpretation this fact implies that the amount of chemical agent mobilized by a nerve impulse decreases considerably for successive impulses, unless they are separated by intervals of about 1 min.

The experiments illustrated in figure 6 may then be explained as follows. The conditioning impulses, particularly the first one, liberated a chemical agent which tended to increase the electrical excitability of the test fibers. If the test shocks were falling at a time when the conditioning volleys were depressing the excitability of the test fibers by some other mechanism, then the enhancing

effects of the chemical agent were neutralized or overcome. As soon as the test shocks fell outside a depressed period, either because the conditioning volleys were stopped, or because their timing with respect to the test shocks was altered, then the increasing action of the chemical agent revealed itself by a prompt rise of response.

It should be pointed out that the increments of test responses in figures 5 and 6, although determined by impulses in the conditioning fibers, are probably not due exclusively to the influence of those conditioning fibers, but are reinforced by events in the additional test fibers recruited during the increment. Thus if an impulse in a previously inactive conditioning fiber can influence the adjacent test elements, the impulses in the previously inactive test fibers which are revealed by the increment of test responses should also exert an influence on the remaining inactive elements.

The question arises why, in appropriate experimental conditions, the test responses do not always remain large (p. 662) after they have increased, unless the test stimuli are stopped for a time sufficient for the dissipation of the responsible chemical agent. This question is readily answered by the previous inference that the amounts of chemical agent released by successive nerve impulses decrease considerably. The maximal height reached at the peak of the increment depends on the concentration of the agent attained by the combined contribution of the conditioning fibers and of the recruited test elements. Indeed, it is obvious that only some of the conditioning fibers were previously inactive, for the test electrodes were in contact with them, as well as with the recording axons, and were activating a certain fraction of both throughout the observations. After the peak concentration has been obtained further contributions by the successive test impulses are small and the rate of production can thus be less than the rate of dissipation, although the rate of production was greater during the period of increment.

Stimulation of nerves leads to the liberation of K ions (see for references Fenn, 1940), and of acetylcholine or adrenaline (see Lissák, 1939). These substances were tested, therefore, as reported in section C (p. 664). Acetylcholine and adrenaline deserve no comment, since they had no appreciable effects. Injections of K Cl, on the other hand, reproduced satisfactorily the effects observed from nerve volleys over conditioning fibers. In addition, the action of K ions was sensitized by veratrine (p. 665), much as the action of conditioning impulses (p. 661). The sensitization by veratrine of the effects of K Cl on nerve is in harmony with the observations of Bacq (1939), who found a similar increase by veratrine of the action of K ions on striated muscle.

It may be inferred tentatively, therefore, that the liberation of K ions is a mechanism by which myelinated fibers in nerve trunks may interact chemically. The mode of action of this potassium shift and the possibility of other chemical influences require further experiments for elucidation.

D. In 1882 Hering reported that activity of some axons could excite neighboring fibers at an injured region in a nerve trunk. This observation was confirmed by Renshaw and Therman (1941). In their experiments the initiation of

the impulses in the recording fibers took place 0.1 to 0.3 msec. after the arrival of the initial impulses to the injured region. Activity of some axons may also initiate nerve impulses in adjacent fibers at regions distant from an injury, but whose excitability is increased by applications of d.c. of appropriate polarity and intensity (Rosenblueth, 1941). The latency for the appearance of the secondary after the arrival of the primary nerve impulses to the hyperexcitable region is from 1 to 2.5 msec.

The discrepancy of latencies suggests that the mechanism of the Hering phenomenon differs from that responsible for my observations—i.e., that the two phenomena are different. In Hering's experiments stimulation occurred shortly after the peak of the initiating spike. The enhancement of excitability in adjacent axons which occurs at that time (fig. 3) is particularly prominent near an injured segment (p. 660). According to the explanation of interaction adopted above (p. 666) the secondary nerve impulses were initiated electrically. In my observations (1941) stimulation probably occurred during the late rise of excitability illustrated in figure 3. Far from an injury the earlier periods of enhancement are only slight (p. 660) and neither cathodal nor anodal polarization modifies interaction (fig. 4). The mechanism of activation was probably chemical instead of electrical.

The data reveal that a nerve trunk is not an aggregate of entirely independent units but that it constitutes a loosely coupled system. Activity of some elements influences the properties of the adjacent units. Since interaction has been shown so far to modify only excitability, that is, the initiation of nerve impulses, and since in "physiological" conditions nerve impulses are not initiated along axons, but only at neuron terminals, it is unlikely that interaction is of importance in these "physiological" conditions. It is of importance, however, for the understanding of the properties of axons and of the mechanism of conduction of nerve impulses—i.e., of the physiology of axons.

SUMMARY

In cats, decerebrated or anesthetized with dial or nembutal, the changes of electrical excitability in some axons (stimulating electrodes *T*, recording electrodes *R*; fig. 1) were studied during and after the passage of nerve impulses in other axons of the peroneal nerve (conditioning electrodes *C*, fig. 1). Changes of excitability at the tested region occurred before, during, and after the arrival of the conditioning volleys (figs. 2 and 3). These changes were not modified by anodal or cathodal polarization of the stimulated tested region (fig. 4). After injections of veratrine, single (fig. 5) or repetitive (fig. 6) conditioning volleys caused a prolonged, usually reversible, increase of the test responses. Injections of KCl, but not of adrenaline or acetylcholine, duplicated the effects of conditioning volleys after veratrine (p. 664).

In the discussion, the results are compared with previous observations on interaction of axons (p. 665). It is inferred that a conditioning volley affects neighboring axons both electrically (early changes, p. 666) and chemically (late changes, p. 667). It is suggested that K ions might be one of the chemical agents

involved (p. 668). It is pointed out that stimulation of axons by impulses in adjacent fibers may be caused by electrical stimulation (Hering phenomenon) or else by a chemical activation (Rosenblueth, 1941; p. 669).

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METABOLISM OF RADIO-IODINE IN THE THYROIDS OF RATS EXPOSED TO HIGH OR LOW TEMPERATURES

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There is general agreement regarding the increased activity of the thyroid in animals exposed to cold, and the reduced activity of this gland in animals exposed to heat. According to results obtained by an indirect method, the release of thyroxine from the thyroid is augmented in the cold and decreased in the heat (1). In the present work, the activity of the gland in animals exposed to extreme temperatures was estimated with the help of radioactive iodine.

METHODS. The radio-iodine used contained mostly the isotope I^{132} with a half-life of 8 days, and was prepared as sodium iodide with some inactive iodide added as carrier. It was decided to use a carrier since the uptake of carrier-free iodine by the thyroid varies with the level of inorganic iodine in the blood at the time of injection, and is therefore not strictly indicative of the activity of the gland. The amount of iodine to be added as carrier should be so great that, when distributed through the body fluids, the previous inorganic iodine blood level becomes negligible. On the other hand, the magnitude of the dose should not be such as to affect markedly the physiological metabolism of iodine, in order that the radio-iodine may retain its rôle as an indicator. Since it did not appear possible to find a dose which would satisfy these two requirements, it was decided to use both a small (0.2 microgram) and a large (5 microgram) dose of radio-iodine per rat. The small dose should have a physiological behaviour. The large dose should overcome completely the variations in the blood iodine level, so that the uptake by the thyroid would be influenced only by the activity of the gland at the time of injection.

In a preliminary experiment, 7 groups of 5 male rats each weighing from 100 to 130 grams were kept either in a cold room at 4°C. or in an oven at 31°C., the controls being at a temperature which varied from 22° to 26°C. The animals were sacrificed 18 hours after an intraperitoneal injection of 0.2 microgram of labeled iodine. The thyroids were left for several days in a 1 N NaOH solution, in which the radioactivity was estimated directly (table 1).

In the main series of experiments, male rats of 150 to 200 grams were kept either in a cold room with the temperature varying from 0° to 2°C. or in a ventilated oven at 32° to 34°C. The temperature of the room in which the controls were kept varied from 19° to 25°C. A dose of 5 micrograms of radio-iodine was injected by the intraperitoneal route. Some of the animals were sacrificed two or in a few cases, one and a half hours after the injection; all, however, being referred to below as the 2-hour group. Another group of animals was sacrificed 30 hours after the injection. The separation of the iodine fractions was carried out ac-

according to the general principles of the micro-method described by Foster (2), all the operations being performed in a 15 cc. Pyrex centrifuge tube. The fresh thyroids were finely ground in about 200 mgm. of sand with the help of a beaded stirring rod. The inorganic iodine was extracted according to the method of Gutman and colleagues (3), drying, however, being limited to about 12 hours. All the fractions were placed in 1 N NaOH for determination of the radioactivity.

In the accompanying tables, the radio-iodine content of the thyroid was expressed as percentage of the injected dose. In tables 2 and 3 comparison of the total radio-iodine values in control and treated animals showed a statistically significant difference, except for the two groups of animals exposed to cold during 40 days (table 2).

In all experiments, a few animals were sacrificed only for histological examination of the thyroids.

TABLE 1

Effect of exposure to cold or heat for various periods of time on the uptake of radio-iodine by the thyroid, expressed as percentage of the injected dose (0.2 microgram), retained 18 hours after injection

	AVERAGE THYROID WEIGHT	PERCENTAGE OF INJECTED IODINE IN THYROID (\pm STANDARD ERROR)	DIFFERENCES FROM CONTROLS (\pm STANDARD ERROR)	RADIO-IODINE CONCENTRATION IN MG. PER 100 GM. OF FRESH THYROID
	MG.			
Controls.....	10	14.3 \pm 2.1		0.29
1-day cold.....	11	12.1 \pm 1.7	2.2 \pm 2.7	0.22
3-day cold.....	11	11.0 \pm 2.0	3.3 \pm 2.9	0.18
7-day cold.....	12	7.5 \pm 0.6	6.8 \pm 2.2	0.12
1-day heat.....	8	8.3 \pm 1.3	6.0 \pm 2.5	0.22
3-day heat.....	7	7.9 \pm 2.1	6.4 \pm 3.0	0.22
7-day heat.....	9	8.7 \pm 1.2	5.6 \pm 2.4	0.17

RESULTS. *Effects of low temperature on iodine fixation by the thyroid.* In the preliminary experiment, carried out with a dose of 0.2 microgram of radio-iodine, the thyroids of the refrigerated animals fixed less radio-iodine than those of the controls (table 1). The iodine uptake appeared to decrease as the length of exposure to cold was prolonged; however, the decrease was statistically significant only in the group exposed to the low temperature for 7 days. Parallel histological examination of the thyroids in other animals kept under the same conditions showed clearcut signs of stimulation only in the 7-day group.

With the higher dosage, the results obtained 2 hours after injection (table 2) revealed an increase in the amount of iodine fixed by the thyroids of the animals exposed to cold. This increase was apparent following a 7-day exposure, maximal after a 26-day exposure, but absent after a 40-day exposure (fig. 1). In addition, the amount of newly formed thyroxine was greater in the glands of the refrigerated animals. Thus in the 20-day group, 10 per cent of the labeled iodine was in the form of thyroxine as compared to 6 per cent in the controls.

Thirty hours after injection, the thyroids of the 7-day group contained less radio-iodine than those of the controls (table 2, fig. 2). However, the proportion of labeled iodine present as thyroxine was greater in the cold group (18 per cent) than in the control animals (13.5 per cent).

TABLE 2

Effect of exposure to cold for various periods of time on the uptake and metabolism of radio-iodine in the thyroid, expressed as percentages of the injected dose (5 micrograms)

LENGTH OF EXPOSURE TO COLD		NO. OF ANIMALS	TOTAL THYROID I	INORGANIC I	DIODOTY-ROSINE I	THYROXINE I
Two hours after injection						
7 days.	Controls.....	10	0.74	0.54	0.17	0.03
	Cold.....	10	1.37	0.92	0.41	0.05
26 days.	Controls.....	5	0.81	0.51	0.25	0.05
	Cold.....	5	2.21	1.18	0.81	0.22
40 days.	Controls.....	12	0.96	0.46	0.44	0.06
	Cold.....	5	0.93	0.43	0.45	0.05
Thirty hours after injection						
7 days.	Controls.....	10	4.36	1.67	2.10	0.59
	Cold.....	11	2.73	1.06	1.17	0.50
40 days.	Controls.....	6	3.10	0.95	1.55	0.60
	Cold.....	5	2.69	0.90	1.24	0.55

TABLE 3

Effect of exposure to heat for various periods of time on the uptake and metabolism of radio-iodine by the thyroid, expressed as percentage of the injected dose (5 micrograms)

LENGTH OF EXPOSURE TO HEAT		NO. OF ANIMALS	TOTAL THYROID I	INORGANIC I	DIODOTY-ROSINE I	THYROXINE I
Two hours after injection						
7 days.	Controls.....	10	0.74	0.54	0.17	0.03
	Heat.....	5	0.51	0.28	0.21	0.01
26 days.	Controls.....	5	0.81	0.51	0.25	0.05
	Heat.....	6	0.45	0.30	0.14	0.01
Thirty hours after injection						
7 days.	Controls.....	5	2.30	1.07	1.07	0.15
	Heat.....	5	2.58	1.33	1.13	0.13

Histological studies showed a clearcut stimulation of the thyroid with thickened epithelium and more basophilic colloid in 5 of 7 animals exposed to cold for 7 days, and in 4 of 5 animals exposed to cold for 26 days. In two of the latter group, the stimulation was intense, with high epithelium and very little colloid.

In 5 animals refrigerated for 40 days, signs of stimulation were doubtful, and overshadowed by a marked increase in the amount of colloid causing distention of many of the follicles.

Effects of high temperature on iodine fixation by the thyroid. The preliminary experiment (table 1) showed a statistically significant decrease in iodine fixation in all groups of rats exposed to heat, although histological examination showed no clearcut changes in the structure of the gland.

Two hours after injection of the large dose of radio-iodine (table 3), the amount of iodine fixed by the thyroids of treated animals was slightly but definitely less than that of the controls. Thirty hours after the injection, it was greater in the treated than in the controls. Histologically, a small reduction in the height of the epithelium was apparent in most of the animals.

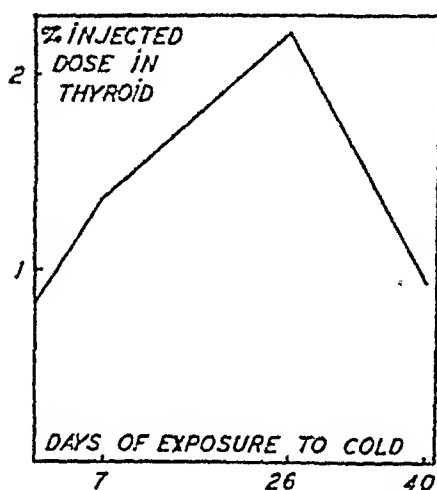


Fig. 1

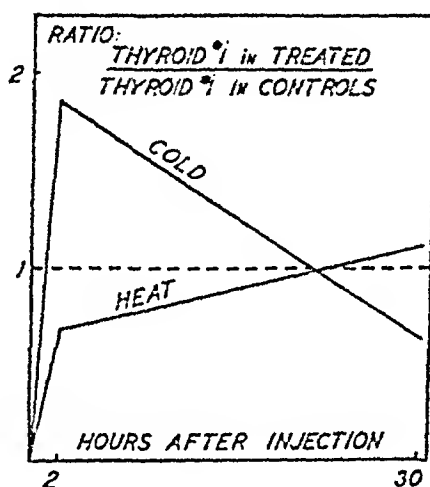


Fig. 2

Fig. 1. Variation in the amount of radio-iodine found at 2 hours after injection, after exposure to cold for various lengths of time.

Fig. 2. Variations in the ratio of the percentage of injected iodine entering in the thyroids of the treated animals over that in the controls, as found in the groups exposed to cold or heat for 7 days. The dotted line may be taken as representing the control values.

DISCUSSION. During the 2 hour period following injection of labeled iodine, the thyroids of the animals exposed to cold for 7 and 26 days fixed respectively 1.85 and 2.70 times as much radio-iodine as the controls. Conversely, the glands of animals kept at a high temperature incorporated less iodine than the untreated, the 7-day group fixing 0.69, and the 26-day group, 0.55 times the control values. On the assumption that there will be little excretion of fixed radio-iodine in the two hours following injection, these iodine ratios of experimentals to controls represent an index of variation in the iodine fixing ability of the thyroid. Histological evidence of activity and inactivity corresponded to the high and low ratios respectively.

Eighteen or thirty hours after the injection of radio-iodine, smaller amounts of labeled iodine were found in the thyroids of the cold groups as compared with the controls. This finding suggested an overall acceleration of thyroid metabolism

in the cold, with increased fixation of iodine (apparent at 2 hrs.) and more rapid excretion (apparent at 30 hrs.). This would be consistent with the increased turnover of thyroxine shown by the greater proportion of this substance found in the thyroids of the cold group. Further, the finding of basophilic colloid may be taken as indirect evidence of increased excretion (4).

In relation to the excretion of thyroxine, Dempsey and Astwood (1) have estimated that the daily output of thyroxine was 9.5, 5.2 and 1.7 micrograms after a 14-day exposure at 1°C., 25°C. and 35°C. respectively. From these figures, we calculated that the excretion of thyroxine at the low temperature was 1.82 times that at the control temperature of 25°C.; and similarly thyroxine excretion at 35°C. was 0.33 times that of the controls. These values for thyroxine release were comparable to those reported above for iodine fixation. This correspondence is again indicative of an overall acceleration of thyroid metabolism in the cold.

This interpretation implies an increased excretion of thyrotropic hormone by the pituitary gland. However, direct stimulation of the thyroid by a thyrotropic extract in the guinea pig (5) resulted in increased iodine fixation at both 2 and 26 hours after injection. Our finding of a decreased fixation in the cold at 30 hours after injection differed from the above result, possibly because of the difference in experimental conditions, e.g., species and dose, or because the cold stimulus acted to some extent directly on the thyroid, resulting in a deviation of iodine metabolism from that produced by the action of the pituitary. However, the separations (table 2) did not reveal disturbances in the general pattern of iodine fixation and metabolism. For example, treated as well as control animals took up a greater percentage of the injected iodine after administration of a small dose (table 1) than after injection of a large dose of radio-iodine (table 2), (6, 7, 8). Similarly, in both control and treated animals given the 5-microgram dose, a high proportion of the iodine was found in the inorganic fraction at the 2-hour period only, indicating that at all environmental temperatures iodine had entered the gland in the form of its ion, as occurs after the injection of rather large doses of iodide (7, 8, 9).

The results obtained at high temperatures especially in the case of the 5 microgram dose, were the opposite of those found in the cold; suggesting that they were produced by a reverse action of the factor effective in the cold, probably an overall deceleration of fixation, metabolism and excretion of iodine.

The duration of the exposure to cold affected thyroid activity markedly. In the initial period, the signs of activation appeared very slowly in contrast with other results (10). Then a period of marked stimulation set in, which extended approximately from the 7th to at least the 26th day of exposure. After 40 days, the thyroids fixed and metabolized quantities of iodine similar to the controls, suggesting that the previous activation had receded. A similar finding is on record, as Starr and Roskelly (11), working out the thyroid histology in rats kept at 12° to 17°C., found a stimulation of the gland which decreased markedly when the exposure was prolonged to 56 days. Apparently a state of equilibrium had been reached, reducing the activation of the thyroid into cold.

SUMMARY

Exposure of rats to cold (0° to $2^{\circ}\text{C}.$) for various periods of time produces a thyroid stimulation which is doubtful after 1 to 3 days, definite after 7 days, maximal at 26 days, but absent after exposure for 40 days.

At the time of maximal stimulation by the cold, the fixation of radio-iodine by the gland is 2.7 times that in the controls. Separation of the iodine fractions of the thyroid at various time intervals indicates that the turnover of thyroxine and the excretion of iodized products are increased to about twice the normal rate.

Exposure of rats to heat (32° to $34^{\circ}\text{C}.$) lessens thyroid activity. This decrease can be observed as early as one day after the beginning of the exposure and persists for at least 26 days. However, this reduction in activities is slight, whether iodine fixation, thyroxine turnover, or excretion of iodized products are considered.

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CARDIAC OUTPUT AND TOTAL PERIPHERAL RESISTANCE IN POST-HEMORRHAGIC HYPOTENSION AND SHOCK¹

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The anesthetized dog when submitted to a prolonged period of drastic hypotension, achieved by controlled bleeding, enters into a state of irreversible shock. The standard procedure developed in this laboratory for producing this state (1) entails lowering the arterial blood pressure by rapid bleeding to 50 mm. of Hg and sustaining it at this level for 90 minutes; it is then reduced to 30 mm. of Hg, at which level it is maintained for an additional 45 minutes. At the conclusion of this 135 minute interval of severe hypotension, all withdrawn blood is rapidly reinfused. Despite the latter procedure, very few dogs have actually survived. Studies have also been conducted by members of this laboratory (2) to ascertain the nature of the permanent derangement produced by severe hemorrhagic hypotension. Both peripheral and cardiac events were followed during the development and terminal stages of the shock state. The cardiometric method which they used to measure cardiac output, though more satisfactory from many standpoints for this purpose than gasometric methods, unfortunately necessitated the employment of open-chest animals. It is therefore hazardous to apply these results to interpret similar situations in the intact animal, since the circulatory state of open-chest animals is extremely labile and unpredictable.

It is now possible to obtain reasonable and consistent cardiac output measurements rapidly and frequently in the *intact* dog by employing the "modified Stewart method," the merits of which have been recently discussed by one of us (3). Employing this more favorable method, the authors have examined cardiac output and total peripheral resistance (TPR) changes which occur during the development and course of "hemorrhagic hypotension" shock in *intact* anesthetized dogs. Evaluations of total peripheral resistance, which is dependent upon accurate measurement of both cardiac output and mean arterial blood pressure, were computed from the equation
$$TPR = \frac{P_m \times 1332}{\text{cardiac output/sec.}} = \frac{\text{dynes} \cdot \text{sec.}}{\text{cm.}^5}$$
 and are expressed as absolute units (A.U.).

PROCEDURE. Following a small preparatory injection of morphine, the dogs were anesthetized with either sodium barbital (175 mgm./kgm.) or chloralose (75-80 mgm./kgm.). The inconsequential operative procedures required for measurement of cardiac output and for recording arterial pressure pulses and mean atrial pressures were then performed. Systemic heparinization⁴ of the dogs

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was instituted to preclude possible interference with output determinations by clotting of blood either in the detection cannula or in the blood samples withdrawn.

Once circulatory equilibrium was established, three consistent and reasonable control values for cardiac output were procured in successive determinations. Immediately thereafter, rapid controlled bleeding was instituted. Sixteen dogs were used. Of these, six died of cardio respiratory failure during the *30 mm. period* of hypotension and therefore only yielded results for the early periods.

One can observe from the graphs in figures 1, 2 and 3 the standard procedure which was adopted for the times at which various cardiovascular events were evaluated. More frequent determinations of the latter did not add significantly to the results. Accurate estimates of the heart rate were obtained from the arterial pressure pulses which were photographed during each cardiac output determination. Mean right atrial pressures were read from a damped saline manometer just prior to each cardiac output measurement. In order to obtain a rough estimate of changes in blood viscosity, hematocrit readings were made on control blood samples collected for output determinations. In most experiments, the right atrial-femoral artery circulation time was measured by a procedure described in a previous paper (3).

RESULTS. Of the six dogs which succumbed from cardio-respiratory failure during the *30 mm. period*, two revealed a severe broncho-pneumonia upon autopsy, whereas the other four showed intense congestion, numerous petechiae and free blood in the lumen of the upper intestine. The cardiovascular changes in these animals during the *50 mm. period* were not unlike those displayed in the other ten animals; hence they are included in the data presented.

With few exceptions, the course of events during the development and terminal course of "shock" is fairly represented by the typical charts of two experiments in figures 1 and 2. The more important exceptions concern the peripheral resistance changes illustrated in figure 3 which will be discussed later. The response of the dogs following complete reinfusion of all withdrawn blood was favorable, yet they all succumbed within ten hours. The impression was gained that this eventual outcome might have been prolonged, but not prevented by any therapeutic measure. The autopsy findings strengthened this viewpoint. There was intense congestion of the upper intestine and frequently of the colon and rectum in every animal, but one. In most instances, large quantities of free blood were found in the lumen of the large and small intestine. In addition, both ventricles and atria usually revealed numerous petechiae throughout the subendocardial surfaces of their walls. Hemorrhages about the base of the valves were not uncommon. Occasionally, small hemorrhagic infarcts were encountered in the lungs. It seems likely that systemic heparinization may have accentuated these findings.

Control blood pressures in ten of the dogs were in a hypertensive range (140 to 170 mm. of Hg); in others, they varied from 95 to 135 mm. of Hg. By variable degrees of reduction of their original blood volumes, all dogs were subjected to a standard degree of hypotension. Five of the dogs which survived the *30 mm. pe-*

riod were originally in the hypertensive category; the other five were in the essentially normal blood pressure group. No correlation between the initial blood pressure level and the post-reinfusion survival time was established.

The recovery of blood pressure immediately after rapid reinfusion of withdrawn blood was so satisfactory that an auspicious prognosis might have been made for most of these animals at this time, if blood pressure level alone were considered. Control levels were regained in five dogs, satisfactory but not pre-existing hypertensive levels were attained in four others; whereas in one dog, a poor response of 54 mm. Hg was obtained. The latter died very suddenly between the first and second post-reinfusion hours. In most cases, the prognosis took an inauspicious turn within an hour after reinfusion; the blood pressure began a progressive decline toward critical hypotensive levels. On two occasions, however, the reinfusion blood pressure level was essentially maintained for 2 to 3 hours, then descended quite rapidly toward critical level.

The cardiac output and the stroke volume were severely reduced throughout the 50 and 30 mm. periods as a result of the great reduction in blood volume which was required to lower blood pressures to these levels. The stroke volume was further embarrassed by cardiac acceleration which abbreviated the cardiac filling time. The cardiac outputs during the prolonged hypotension varied from 29 to 45 per cent of the original control volumes. Only minor fluctuations were observed during this 135 minute interval. Concordant with the development of hypotension and the large reduction of minute volume, there was a marked prolongation of the circulation time; in many instances it was doubled (fig. 1). Even though generally tachycardic during the control period, heart rates were further accelerated during the 50 mm. period. During the 30 mm. period they were more variable; in some cases the heart rate was further accelerated, in others, slightly decelerated, in still others even retarded below control values (figs. 2 and 1). With the latter slowing, stroke volumes increased somewhat without any significant change in the cardiac output. Hematocrit values decreased progressively during the 50 mm. period and remained constant during the 30 mm. period. This connotes hemodilution and a reduced blood viscosity.

The response of cardiac output to the reinfusion of withdrawn blood was not as auspicious as the blood pressure recovery. In six dogs it was very satisfactory, the cardiac outputs returning to 90 to 118 per cent of control volumes. In the other four, it was mediocre at best, varying from 45 to 85 per cent of the control volumes. During the next three hours, however, the cardiac output in seven dogs diminished rapidly. Following this it usually stabilized at low values similar to those established during the hypotension period (fig. 1), where it remained until death occurred. In three animals, cardiac output was only slightly diminished during the first 2 to 3 hours after reinfusion and then decreased abruptly to similar low volumes.

The stroke volume was improved after reinfusion, but never regained control values in nine of the dogs. In eight animals, it was progressively reduced thereafter, much as the minute volume. In two dogs, however, stroke volume improved as the post-reinfusion period progressed. This was related to a simul-

taneous progressive slowing of the heart in these two instances. In the eight animals mentioned above, the heart rate regained or approached normal rates immediately after reinfusion and underwent a secondary acceleration during the initial hours of the post reinfusion period. This acceleration was sustained until death in five of the dogs (fig. 2); in the others, even if they underwent this secondary acceleration, the heart was markedly slowed and irregular during the terminal hours of the experiment (fig. 1). In all but one animal, a tendency toward progressive hemoconcentration was in evidence throughout most of the

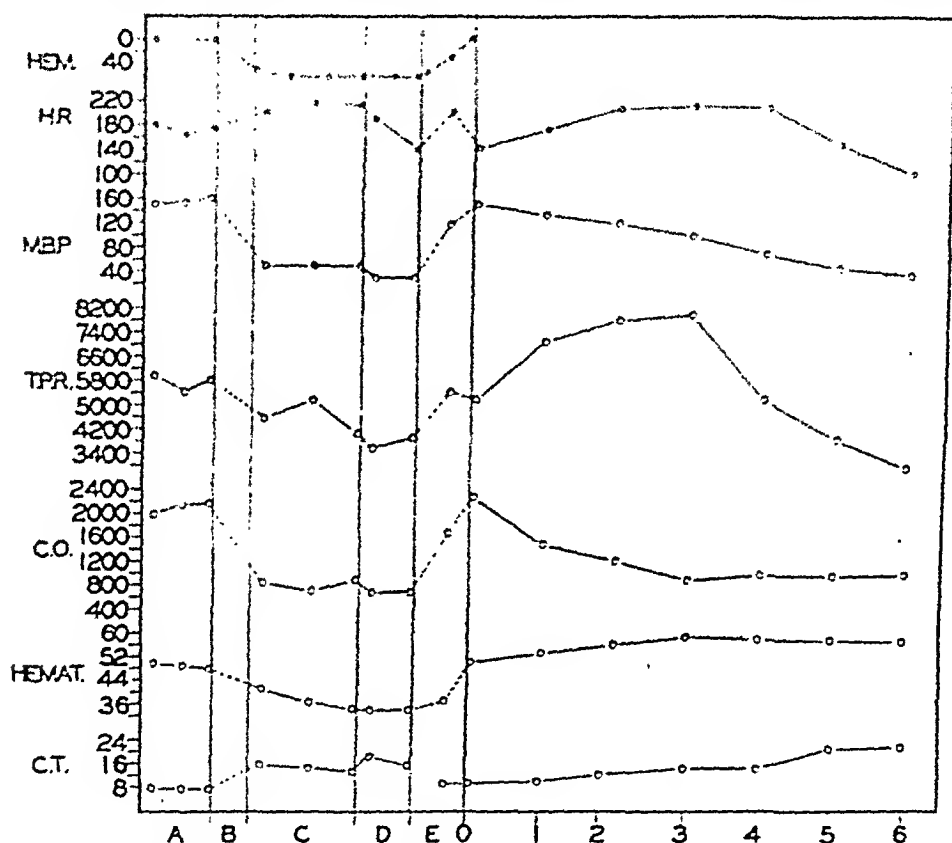


Fig. 1. Plot showing (Hem.) blood withdrawn and reinfused in ml./kilo; sequence of cardiodynamic events (*H.R.*, heart rate; *M.B.P.*, mean arterial pressure in millimeters of mercury; *TPR*, total peripheral resistance in absolute units; *CO*, cardiac output, ml./min.; *Hemat.*, hematocrit readings; and *CT*, circulation time in seconds during (A) control period; (B) hemorrhage; (C) 50 mm. of Hg hypotension; (D) 30 mm. of Hg hypotension; (E) reinfusion; and hourly thereafter.

post-reinfusion period. The circulation time, though essentially normal after reinfusion, again steadily increased until the circulation again assumed the sluggish characteristics seen during the hypotension period (fig. 1).

The changes in peripheral resistance were rather variable. The types of changes which are likely to be encountered are illustrated in the charts of figures 1, 2 and 3. Two different responses were observed during the 50 mm. period. In eight of the dogs, *TPR* was elevated above respective control values during the greater part of the period, as shown in figures 2 and 3, II. In the other eight

animals, TPR was markedly reduced at the onset of the 50 mm. period. In five of these, however, TPR increased, approaching control values as the period progressed. Regardless of the response during this period, all TPR values were reduced below their respective control values during the 30 mm. period. Those with an elevated TPR during the 50 mm. period showed the greatest reduction. At the termination of this hypotension period, TPR values varied from 64 to 84 per cent of their respective control values.

Immediately after reinfusion, TPR in five animals varied from 95 to 116 per cent of their respective control values; in the others, reasonably satisfactory though definitely subcontrol values were attained (60 to 92 per cent of control). The course of the TPR during the remainder of the post-reinfusion period was extremely variable: 1. In four dogs, represented in figure 1, the TPR rose progressively to a peak value high above the control within the first 2-3 hours after

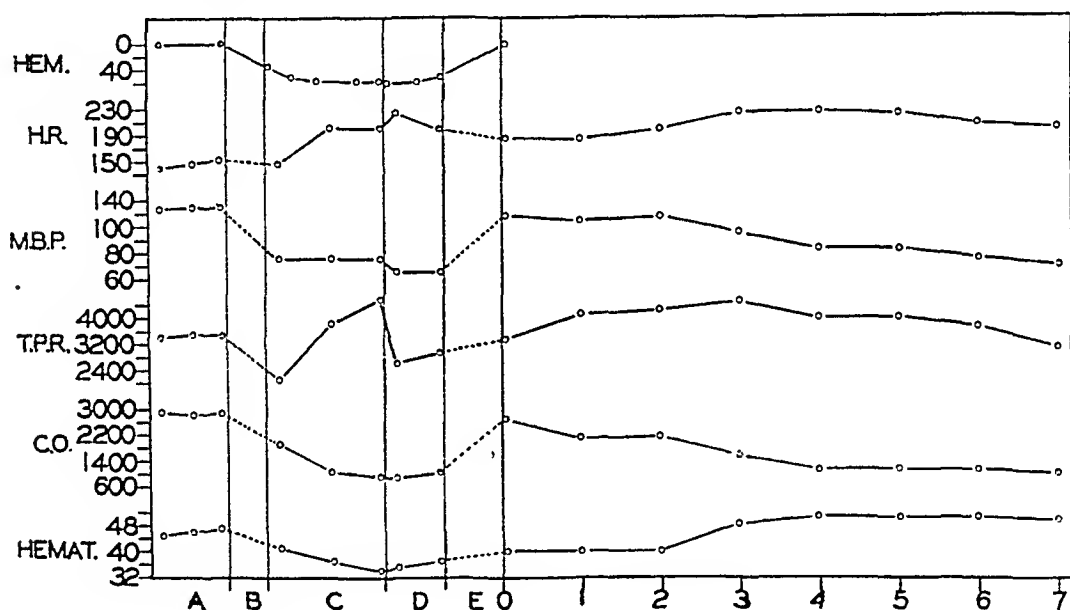


Fig. 2. Same as figure 1.

reinfusion; it then subsided suddenly to markedly subcontrol values within the succeeding 2 to 3 hours prior to the death of the animal. This response is also represented in figure 3, II. 2. On two other occasions the TPR ascended more gradually to a level above the control and then diminished to a level not far below the respective control at about the same rate that it ascended. This is exemplified by the TPR changes in figure 2. 3. As shown in figure 3, III, TPR hovered around the control values for approximately five hours, suddenly rose to a peak (135 per cent of control value) from which it sank rapidly to a subcontrol level (71 per cent of control) much as in the animals in the first group above. In this particular animal the cardiac output never stabilized at a low volume but continued to diminish at a more gradual rate until the experiment terminated. 4. On two occasions, typified by figure 3, I, TPR failed to attain the control value after reinfusion; it subsided rapidly from this reinfusion level to subcontrol levels

until death occurred within three hours. *5.* The final type (fig. 3, IV) resembles that seen in figure 3, III in that TPR changed very little for 5 to 6 hours after essentially normal values had been regained following reinfusion. In this animal, however, TPR did not rise belatedly; its peak value (116 per cent of control) was gained immediately after reinfusion. At about the sixth hour after reinfusion, it diminished fairly rapidly to a markedly subnormal level (64 per cent) just prior to death. Peculiarly, this was the only animal in which autopsy findings were completely negative throughout.

Just prior to death, TPR was but slightly reduced in five of the dogs, the values varying from 88 to 102 per cent of their respective control values. An example is shown in figure 2. In the other five it was severely reduced, the values recorded just prior to death varying from 40 to 71 per cent of their respective controls (example, fig. 1).

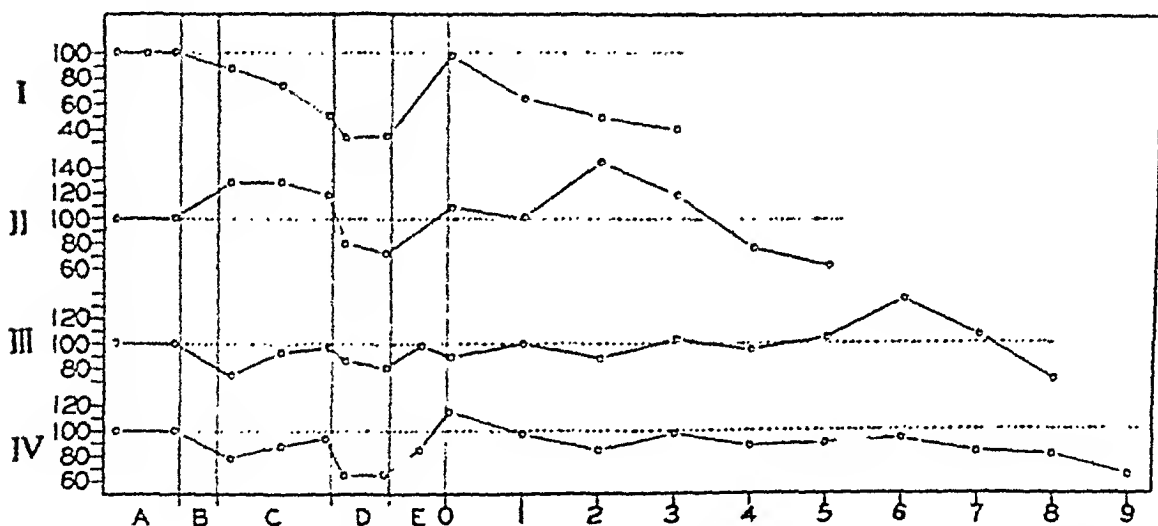


Fig. 3. Types of TPR curves encountered during post-hemorrhagic hypotension; during reinfusion and circulatory failure. Ordinates in percentage changes from control = 100 (dotted lines). Abscissal, indications as in figure 1. Discussion in text.

DISCUSSION. An analysis of the cardiac output and TPR changes, as well as of the changes in related events, permits fairly definite conclusions as to the nature of the primary derangements incurred from these drastic hemorrhagic hypotension procedures.

The total resistance to the outflow of blood from the aorta is governed by several factors: 1. Fundamentally, it depends upon the physical dimensions of the vascular system; hence the latter largely determines the range of TPR fluctuation which may result from superimposed influences. These dimensions may be considerably altered with growth and ageing processes. 2. It is also dependent upon the effective viscosity of the blood, decreasing as the cell/plasma ratio decreases and *vice versa*. 3. Finally, TPR is continuously modified by functional modifications in the caliber of minute vessels induced by *a*, vasomotor nerve influences; *b*, action of chemical agents, and *c*, variable extra-vascular ten-

sions created by muscle tone, tissue fluid pressure and the like. It is more often the rule than not that several of these factors are simultaneously involved in circulatory reactions and it is important to evaluate the relative contributions of each whenever possible. In these studies, it has been possible to infer the dominating factor with a high degree of probability.

Since the characteristic of irreversibility develops during the hypotension period, the course of events during the *50* and *30 mm. periods* must be carefully examined and analyzed.

a. Fifty millimeter period. Among the anticipated responses to lowering of the blood pressure by hemorrhagic procedures which were actually observed in these dogs may be included *a*, an accelerated heart rate; *b*, a diminished stroke and minute volume; *c*, a prolongation of the circulation time; *d*, a reduction of venous or atrial pressure, and *e*, a progressive hemodilution. The nature and causes of TPR changes in the development and course of shock have not been established.

These experiments have demonstrated that TPR *a*, may progressively decrease, as in figures 1 and 3, I; *b*, may fall initially and then recover toward control values, as in figures 2, 3, III and IV, or *c*, may increase, as in figure 3, II. In attempting to determine the factors which dominate these TPR changes, it is initially expedient to eliminate those TPR determinants which change uni-directionally and to much the same extent in all animals, for they cannot be dominant forces in such variable reactions. Little can be said about the extravascular forces and their effects upon venous return. There is, however, little reason to anticipate that these forces are significantly or variably altered and hence that they play a major rôle in the reactions of these supine anesthetized animals. Furthermore, comparison of the hematocrit values obtained during this period with related curves published by Whittaker and Winton (4) convinced the authors that within the ranges of cell/plasma ratios found in these experiments, the effective viscosity in the blood vessels of the body is not greatly reduced. Since the hematocrit readings were reduced to essentially the same extent in different experiments, it is believed that its effect in reducing TPR does not differ much in the various animals. It is probable, therefore, that no great error is incurred in the assumption that the above factors remain fairly equal in different experiments and that they play, at most, a very small rôle in determining changes in TPR. Consequently, the variable TPR changes are chiefly due to active changes in the caliber of minute vessels, dominantly the arterioles. At the onset of the *50 mm. period*, such changes are not likely to be caused by a peripheral accumulation of metabolic products. As the period terminates, however, such products may very well tend to counteract or even overbalance the influence of vasomotor nerve impulses. Chemical influences certainly cannot be very significant in those animals in which TPR was elevated far above control values. Therefore, the different TPR trends during *this period* are primarily attributable to varying responses of the vasomotor nervous mechanisms to the drastic circulatory upset which has been produced. One might speculate that this variability can be explained upon the differences in the progress of anesthesia in these dogs. The latter is minimized, however, by the fact that hemorrhagic procedures were

never instigated until four hours after the anesthetic had been administered and by the fact that strikingly similar responses were observed in dogs under such dissimilar anesthetics as sodium barbital and chloraloseane. Whatever the reason, it can be stated that vasomotor nervous compensation during the 50 mm. period was very strong in some, moderate and delayed in others and actually inadequate in still other dogs. Regardless of the course of the TPR changes seen during this period, the outcome was eventually the same in all animals, namely, death within 2 to 10 hours after reinfusion of the withdrawn blood.

b. Thirty millimeter period. The reduction of the blood pressure to 30 mm. of Hg was accomplished either by allowing it to fall spontaneously or by a very small amount of bleeding. Hence, cardiac output was not significantly altered. Greater or lesser acceleration of the heart rate was seen in some animals; in others the heart was markedly retarded below control rates. Such slowing was not altered by bilateral vagotomy; hence it probably occurred as a result of the action upon the pacemaker of chemical metabolic accumulants, largely produced by an inadequate coronary flow at these low pressures.

The TPR changes during this period of drastic hypotension were uniform in direction, i.e., TPR fell below normal in every animal. Blood viscosity is not a factor in this decline, inasmuch as the hematocrit values remained essentially unchanged. The TPR changes therefore must be primarily determined by active changes in peripheral vessels. There can be little question that anoxic and accumulated metabolic by-products exert considerable influence upon the peripheral vasculature tending to produce vasodilatation, thus reducing the effectiveness of any existing nervous influences on these same structures. In view of the fact that large vasopressor responses can be induced by pressor nerve stimulation and by asphyxial conditions at this time, it seems evident that the vasomotor centers have not failed. It may be, however, that the intensity of their reactions is lessened which, coupled with the dilating action of anoxia and metabolites, brings about a reduction in the total peripheral resistance.

c. Post-reinfusion period. The return of arterial blood pressure immediately after rapid reinfusion of withdrawn blood to satisfactory levels was an auspicious beginning. However, the cardiac output was satisfactorily improved in only six of the ten dogs. Immediately thereafter, cardiac output began to decline and became progressively smaller until volumes equivalent to those encountered during the hypotension period were again attained. Thus, as others have shown in other forms of shock, the declining arterial pressure which follows reinfusion of blood after severe prolonged hemorrhagic hypotension is due chiefly to progressive reduction in cardiac output. But, during the later stages, further decline of arterial pressures occurs without additional reduction of cardiac output, indicating that now peripheral factors are operating. In the terminal stage, hearts of several dogs were considerably slowed and irregular in rhythm, as in the 30 mm. period. This terminated life.

The TPR values obtained immediately after reinfusion were essentially normal or slightly higher. Following this, a variety of changes in TPR were seen, as shown in figures 1, 2 and 3. The interpretation of these varied responses is

somewhat more complicated than that of the changes encountered during post-hemorrhagic hypotension. The cell/plasma ratio remained essentially normal in two dogs; in the others, it increased above the control. This demonstrates that hemoconcentration can occur in circulatory failure following hemorrhage and reinfusion, and that the condition also qualifies as a shock state in accordance with Moon's criterion. However, the hematocrit readings never exceeded 60 and were never less than 40 in controls. Consequently, in accordance with curves of Whittaker and Winton, the changes could have only a minimal effect in increasing the effective viscosity of blood in the blood vessels. It is, however, unlikely that this physical determinant alone is responsible for the marked elevation in TPR which was observed in some experiments, and of course fails to account for reductions. Such changes must involve alteration of vasomotor tone. During the early hours of the *post-reinfusion period* and until a critically sluggish circulation again develops, it is questionable whether anoxia or the accumulation of metabolic by-products are severe. The possibility that vasopressor substances often present in shed blood are concerned is virtually excluded in view of the facts that *a*, such agents should produce a sudden, not a progressive elevation of TPR when the blood is reinfused rapidly, as shown in figures 1, 2 and 3, II; *b*, they cannot be responsible for the delayed response seen in figure 3, III, nor *c*, can they explain the prolonged maintenance of TPR at essentially control levels, as in figure 3, III and IV. Furthermore, attempts were made to prevent the accumulation of vasoconstrictor substances in the shed blood by keeping the latter refrigerated until just prior to reinfusion. Thus, the TPR changes depend upon the reactivity of the vasomotor nerve mechanisms.

During the terminal hours preceding death, however, TPR always falls, in some cases to slightly subcontrol and in others to extremely subcontrol values. That this cannot be due to failure of the vasomotor centers—although the activity of the latter may be somewhat less intense—is signified by the increased TPR which can be induced as a result of asphyxial procedures or upon stimulation of some afferent pressor nerve. Since a significant decline of TPR does not occur until the circulation has again become very sluggish at critically hypotensive blood pressure levels, and since cardiac slowing and irregularity re-occur at this time in some dogs, it seems reasonable to assume that, as during the *30 mm. period*, humoral or metabolic agents are again more effectively overcoming the nervous influences upon blood vessels in the periphery, thus affecting a reduction in the effective TPR. The variability in the degree of TPR reduction just prior to death may be supposed to be related to the variable concentration of humoral or metabolic agents and their variable effectiveness in counterbalancing the effects of nervous activity upon the smaller blood vessels.

The method for producing standardized hemorrhagic shock employed in these experiments was arrived at in this laboratory (5) by "trial and error" methods. It was found that shock could be produced more consistently by maintaining standard low arterial pressures for specified intervals of time than by bleeding the animal by definite volumes of blood per kilo of body weight (1).

A physiological basis for such a procedure would be suggested if it could be shown that this correlated with the reduction of the *circulatory index* (cardiac output per square meter of body surface) to a definite minimal value or with a definite percentage reduction of the control cardiac output. Since it is not clear whether, with the intense constriction of surface vessels, such calculations of *circulatory index* are more properly made on a surface area or weight basis, we contented ourselves with the method described. As stated, our results showed that cardiac output was always reduced to 29 to 45 per cent of the original control values during most of the 135 minutes of hypotension. In other words, reduction of the circulatory value to one-half to one-third of the normal output for two and one-quarter hours suffices to induce the irreversible state. However, the considerable ranges in percentage reductions of cardiac output indicate that the simple procedure for producing hemorrhagic shock by holding arterial pressures at specified levels does not result in equal reductions of total blood flow in different animals. This merely illustrates again the difficulty of creating entirely similar conditions in different animals for the study of shock problems. It may also well explain why much shorter periods of less drastic hypotension often suffice to produce shock while even more prolonged periods of severe hypotension occasionally fail to do so.

SUMMARY AND CONCLUSIONS

1. Utilizing a "modified Stewart method" for determining cardiac output, variations of the latter and of total peripheral resistance (TPR) were studied during the course of standardized hemorrhagic shock in relation to other cardiodynamic events and hematocrit changes.

2. During a 90 minute period of 50 mm. of Hg hypotension and a subsequent 45 minute period at 30 mm. of Hg, cardiac output and stroke volume were reduced to 29 to 45 per cent of the control flow. Although they were restored to normal in the majority of experiments immediately after reinfusion, in some the recovery was only to 45 to 85 per cent of control values. During the three hours succeeding reinfusion, cardiac output decreased rapidly and was the chief cause of the declining arterial blood pressure. In the final stages cardiac output stabilized at low levels and the continued fall of blood pressure was occasioned chiefly by peripheral factors. Slowing and failure of the heart was often the ultimate step in the series of cardiodynamic events leading to death.

3. Hematocrit readings indicated a hemodilution during the periods of hypotension and a tendency toward concentration following reinfusion of the blood.

4. The course of events in standardized hemorrhagic shock is therefore similar to that described in other experimental types in that *a*, hemoconcentration occurs, and *b*, progressive reduction of cardiac output is chiefly responsible for the progressive decline of arterial pressures after reinfusion.

5. Despite the universally fatal outcome, changes in the total peripheral resistance were extremely variable during the periods of post-hemorrhagic hypotension and during circulatory failure which developed after reinfusion of blood. The different trends are analyzed. Arguments are advanced that

physical factors concerned in such changes can be evaluated and that an estimate of directional changes in vasomotor tone can be made. Supplementary evidence is cited from which the conclusion is reached that humoral or metabolic factors play a considerable rôle in these changes.

6. In the method for producing shock by holding mean arterial pressures at successive levels of 50 and 30 *mm. of Hg* for specified intervals of time, cardiac output was reduced to 29 to 45 per cent of the original blood flow. Such ranges of reduction indicate that the procedure recommended for the rather regular production of hemorrhagic shock does not result in equivalent reductions of circulatory values when applied to different animals. Of course, the possibility that other factors may enter cannot be excluded.

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THE RENAL AMINO ACID CLEARANCE IN THE NORMAL DOG¹

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It is known that the kidney excretes small quantities of amino acids at normal plasma concentration. Significant increase in the excretion of amino acids occurs at elevated plasma levels but adequate studies to establish the existence of a maximal rate of tubular transfer are not available. It has been suggested (Smith, 1937), that different amino acids are probably excreted by the kidney in specific fashion. Kirk (1936) found that after the administration of glycine in man the amino acid clearance increased as the plasma amino acid level rose and appeared to be independent of diuresis. At low plasma amino acid N concentrations (1-4 mgm. per 100 cc.), l-tyrosine and l-histidine were almost completely reabsorbed from the glomerular filtrate in the dog; however, at slightly higher plasma amino acid N (3-7 mgm. per 100 cc.) N-methyl-l-tyrosine and N-acetyl-l-tyrosine were not reabsorbed so completely (Doty, 1941).

Previous studies in this laboratory (1942) on normal and hypoproteinemic dogs, to which casein hydrolysate was administered, have shown that with plasma amino acid N² levels about twice the fasting value, tubular reabsorption of amino acids is almost complete, so that the amino acid clearance is usually under 1 cc. per sq.m. per min. The experiments reported here were designed to see whether a maximal rate of tubular transfer for amino acid exists.

The elevated plasma amino acid levels produced by oral administration of large quantities of amino acid are often associated with severe vomiting. This, combined with the exceedingly rapid removal of amino acids from the blood stream by the liver and other tissues, makes it difficult to maintain high plasma levels. In the first group of experiments the administration of amino acids by stomach resulted in a sharp rise and fall in the plasma concentration without maintaining an elevated level. Hence the clearances were determined with falling plasma concentration.

EXPERIMENTAL PROCEDURE. *Gavage experiments.* Normal young female dogs were fed a standard complete ration. They were trained to submit to being bled and catheterized. After an 18 hr. fast a preliminary gavage of water was given to insure adequate urine flow. An hour after the gavage a sample of blood was withdrawn, either from the jugular vein in some experiments or from the femoral artery in others,³ the dog was catheterized and returned to the cage.

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² Throughout the paper plasma amino acid N refers to alpha amino acid N as determined by the nin-hydrin method.

³ In our hands on several occasions no significant difference was found in the amino acid N content of venous and arterial blood when withdrawn simultaneously.

Thenceforth the dog was bled and catheterized every half hour and replaced in the cage between bleedings. After two control periods of half an hour each, an amino acid or a mixture, approximately 350 mgm. amino acid N per kilo body weight, was given by stomach tube. About an hour was allowed for absorption of the amino acid and thereafter the urine was collected in periods of 30 min. Attempts to attain still higher plasma levels by administration of a second gavage were defeated by incessant vomiting. Sufficient creatinine was given either by mouth or subcutaneously so that glomerular filtration could be estimated from the exogenous creatinine clearance (Shannon, 1936).

Chemical methods. Minimal amounts of dry potassium oxalate were used as anticoagulant. In a few experiments blood urea N was determined by the Van Slyke urease method (1927). The centrifuged plasma was analyzed for alpha-amino acid N by the Van Slyke ninhydrin method (1941). Creatinine was determined by a modification of the Folin-Wu method (1919) suggested by A. A. Weech (1940); two hours after the addition of the alkaline picrate the color was found to be maximal and stable; the color was measured in an Evelyn photoelectric colorimeter with a filter of 520 millimicrons. Analyses of the urine included alpha-amino acid N, creatinine, and in part of the studies urea N by the same methods. Other procedures have been described previously (1942). For calculation of the renal clearances, concentrations of plasma creatinine, plasma amino acid N, and blood urea N were plotted against time on semi-logarithmic co-ordinates. The mean plasma concentration of each urine collection was obtained by interpolation to the mid-period (Smith, 1937).

RESULTS. Amino acid administered by gavage. Using the technique outlined above the effects of ingestion of dl-alanine, glycine, and casein hydrolysate⁴ were studied. Observations during the control periods (table 1) showed that at normal plasma amino acid levels only minimal amounts of amino acid were excreted in the urine, so that the renal amino acid clearance was less than 1 cc. per sq.m. per min. Glomerular filtration as measured by the exogenous creatinine clearance fluctuated somewhat, apparently independently of urine flow, and the ratio amino acid clearance/creatinine clearance was constant.

When dl-alanine was administered by gavage (table 1—expts. 1 and 2) the plasma amino acid N rose sharply to fourfold the normal value within an hour and then fell almost as sharply. At the high plasma amino acid values, up to 4 times the fasting level, large amounts of amino acid were excreted in the urine and the amino acid clearance ranged from 19 to 44 cc. per sq. m. per min. When glycine was given (table 1—expt. 3) the plasma amino acid N rose to double the fasting value in the first hour and to three times this value during the second hour. At plasma amino acid levels twice the normal, lesser amounts of excreted amino acids resulted in lower amino acid clearances of 8 to 10 cc. per sq.m. per min. With both glycine and dl-alanine a marked increase in the ratio amino acid clearance/creatinine clearance indicated a large increment in the fraction of filtered amino acid excreted.

In contrast, when casein hydrolysate was given (table 1—expt. 4) the plasma

⁴ Kindly furnished by Mead Johnson and Company under the trade name "Amigen."

TABLE 1

Results before and after administration of amino acids by gavage to normal dogs

TIME	URINE FLOW	MG./100 CC.				MAXIMUM CLEARANCE (CC./MIN./SQ.M)		CLEARANCE RATIO A.A. CREAT.	AMINO ACID N (MG./100 CC. OF GLOMERULAR FILTRATE/SQ.M.)			
		Creatinine		Amino Acid N		Creat- inine	Amino Acid		Filtered	Ex- creted	Reab- sorbed	
		Plasma	Urine	Plasma	Urine							
Experiment 1												
minutes	cc./min.											
0	Gavage with 400 cc. water to dog 958 (10.6 kilo, S.A. = 0.5341)											
26	Subcutaneous injection 1.6 grams creatinine in 20 cc. water											
60-89	2.2	13.0	302	3.56	0.5	94	0.6	0.006	3.56	0.02	3.54	
89-118	2.4	11.5	223	3.56	0.3	87	0.4	0.005	3.56	0.02	3.54	
124	Gavage 25 grams dl-alanine in 200 cc. 5% glucose in saline											
128	Subcutaneous injection 3.4 grams creatinine in 40 cc. water											
148-178	1.2	29.8	1351	11.60	139.3	103	27.4	0.266	11.60	3.08	8.52	
178-208	1.1	28.2	1500	11.50	252.1	105	43.2	0.411	11.50	4.72	6.78	
208-238	1.1	23.7	1668	8.65	184.9	145	44.0	0.303	8.65	2.63	6.02	
Experiment 2												
0	Gavage with 600 cc. water and 5 grams creatinine to dog 963 (15.9 kilo S.A. = 0.6771)											
77-107	2.3	15.7	431	4.21	0.3	92	0.2	0.002	4.21	0.01	4.20	
107-139	1.6	14.7	428	4.76	0.4	68	0.2	0.003	4.76	0.01	4.75	
145	Gavage 40 grams dl-alanine and 2 grams creatinine in 400 cc. 2.5% glucose in saline											
139-200	1.4	14.0	700	8.40		100						
200-231*	1.1	13.9	855	15.35		96						
231-261	0.5	13.3	1210	13.80	381.0	63	19.2	0.305	13.80	4.17	9.63	
261-290*	0.8	12.3	1205	14.15		113						
290-321	0.6	11.3	1419	11.00	392.1	104	29.5	0.284	11.00	3.13	7.87	
Experiment 3												
0	Gavage with 300 cc. water to dog 95S (10.4 kilo, S.A. = 0.5299)											
10	Subcutaneous injection 5 grams creatinine in 100 cc. water											
30-60	0.3	36.0	4241	4.08	2.5	59	0.3	0.005	4.08	0.02	4.06	
60-90	0.3	36.6	4751	3.77	2.7	61	0.3	0.005	3.77	0.02	3.75	
91	Gavage 25 grams glycine in 200 cc. 5% glucose in saline											
120-150	0.7	24.4	1818	8.59	51.7	103	8.3	0.081	8.59	0.70	7.89	
150-180	0.8	19.9	1253	10.90	69.1	99	9.9	0.100	10.90	1.10	9.80	

TABLE 1—*Concluded*

TIME	URINE FLOW	MG./100 CC.				MAXIMUM CLEARANCE (CC./MIN./SQ.M)		CLEARANCE RATIO A.A. CREAT.	AMINO ACID N (MG./100 CC. OF GLOMERULAR FILTRATE/SQ.M.)		
		Creatinine		Amino Acid N		Creatinine	Amino Acid		Filtered	Excreted	Reabsorbed
		Plasma	Urine	Plasma	Urine						
Experiment 4											
minutes	cc./min.										
0	Gavage with 400 cc. water and 5 grams creatinine to dog 963 (15.4 kilo, S.A. = 0.6771)										
80-110	1.9	19.6	597	4.45	0.9	85	0.5	0.006	4.45	0.03	4.42
110-140	0.5	15.5	1835	4.45	3.8	82	0.6	0.007	4.45	0.03	4.42
145	Gavage 65 grams casein hydrolysate powder in 400 cc. 2.5% glucose in saline										
140-170	0.2	13.1	3415	6.60		77					
170-200	0.2	11.6	3523	8.63		90					
200-230	0.3	10.4	2868	9.13	15.1	110	0.7	0.006	9.13	0.05	9.08
230-260	0.3	9.4	2118	9.01		100					
260-293	0.4	8.2	1641	8.55		112					
293-323	0.5	7.3	980	8.75	10.6	103	0.9	0.009	8.75	0.08	8.67

* Vomited.

amino acid N level was doubled within half an hour and never rose above that value during the three-hour period of observation. Although a slight increase in urinary amino acid was noted it was not sufficient to alter the amino acid clearance significantly from that obtained in the control periods, and the ratio amino acid clearance/creatinine clearance remained constant.

In the gavage experiments, with approximately equal dosage (calculated as amino acid N) of dl-alanine, glycine and casein hydrolysate, the plasma level attained was apparently governed by factors other than the solubility of the amino acids ingested. Variation in the rate of absorption of different amino acids from the intestinal tract may account for the failure to obtain higher values with casein hydrolysate.

The amino acid clearances described above were determined with falling plasma amino acid concentration. Since the sharp rise and fall of plasma amino acid concentration resulted in failure of maintenance of high plasma levels and since there may be a lag in the adjustment of the renal tubular cells to successively lower plasma levels, the estimation of tubular reabsorption under these conditions is not necessarily accurate. However, the experiments confirmed the fact that with oral administration of the single amino acids or casein hydrolysate, plasma amino acid N levels under 8 to 9 mgm. per 100 cc., were associated with little loss of amino acid in the urine.

In an attempt to maintain high plasma amino acid levels for longer periods, amino acids were given by continuous intravenous infusion. Under these condi-

tions restraint of the animal on the board is inevitable and copious diuresis results. However, more reliable measurement of tubular reabsorption is attained since the clearances are determined at relatively constant plasma amino acid levels with successive increases in plasma concentration.

PROCEDURE. *Continuous intravenous administration.* A preliminary gavage of water was followed by the continuous intravenous infusion of creatinine in 5 per cent glucose in physiological saline at 6 to 11 cc. per min. After diuresis was established, urine was collected by indwelling catheter for several control periods of 5–10 min. Blood was withdrawn just before and just after the control urine collection periods. Then amino acid was added to the infusion mixture without change in the rate of administration; when the plasma amino acid level attained a constant higher value, blood was again withdrawn before and after the urine collection periods. Several experiments on the same dog with one material were necessary to obtain the complete range of clearances at elevated plasma amino acid values. It was technically difficult to maintain constancy of the plasma amino acid concentration at high levels; changes in the rate of infusion even of a few minutes' duration produced rapid fluctuations in plasma concentration. The effect of these fluctuations of plasma concentration on the clearance was minimized by selection of short urine collection periods.

RESULTS. *Continuous intravenous infusion.* To evaluate the physiological effect of the amino acids administered intravenously at so rapid a rate, it was necessary to determine the results of a prolonged intravenous infusion of 5 per cent glucose in saline alone on glomerular filtration, plasma amino acid level, and the renal clearance of amino acids. In a normal dog (table 2—expt. 5) diuresis with progressive decrease in urine flow and a few days later (table 2—expt. 6) with progressive increase in urine flow was produced. Observations over a period of 2 hrs. in each experiment disclosed a slight fall in the plasma amino acid N level and the excretion of very small amounts of amino acid with consequent low amino acid clearances. Fluctuations in creatinine clearance were observed similar in degree to those previously noted in control periods with longer urine collection intervals. In spite of the wide range in urine flow no significant difference was noted in the amount of amino acid excreted and the ratio amino acid clearance/creatinine clearance remained constant.

The same dog (table 2—expts. 7, 8 and 9) was now given dl-alanine by constant intravenous infusion. In two of the studies (expts. 7 and 8) preliminary control periods were followed by observations at a high plasma amino acid concentration, while in the third (expt. 9) the control period was omitted and only successively higher concentrations were attained. As shown in the table, the findings of the control periods agreed with those observed in the previous prolonged studies. With elevated plasma amino acid N levels at least twice the normal, as in the gavage experiments, increasing amounts of amino acid were excreted with correspondingly high renal amino acid clearances. During the control periods with high urine flow at normal plasma amino acid N levels the renal amino acid clearance was uniformly low. At plasma amino acid N levels two to ten times the normal and with a urine flow of 5 cc. per minute, the amino acid clearance was

TABLE 2—*Concluded*

TIME	URINE FLOW	MG./100 CC.				MAXIMUM CLEARANCE (CC./MIN./SQ.M.)		CLEARANCE RATIO A.A. CREAT.	AMINO ACID N (MG./100 CC. OF GLOMERULAR FILTRATE/SQ.M.)		
		Creatinine		Amino Acid N		Creatinine	Amino Acid		Filtered	Excreted	Reabsorbed
		Plasma	Urine	Plasma	Urine						
Experiment 8—Continued											
minutes	cc./min.										
89-104	4.2	12.1	195	3.62	0.6	102	1.0	0.010	3.62	0.03	3.59
104-114	6.2	12.1	160	3.81	0.3	124	0.8	0.006	3.81	0.02	3.79
117	2.0% dl-alanine (0.32% NH ₂ N) added to infusion mixture at 6 cc. per minute										
134-144	6.2	11.8	158	7.89	9.6	125	11.4	0.091	7.89	0.72	7.17
144-154	7.1	11.8	148	8.84	10.3	135	12.7	0.094	8.84	0.82	8.02
Experiment 9											
0	Gavage with 400 cc. water										
45	Continuous I.V. with 0.2% creatinine in 5% glucose in saline at 7 cc. per minute										
67	3.8% dl-alanine (0.60% NH ₂ N) added to infusion mixture at 6 cc. per minute (S.A. = 0.6493)										
106-113	9.0	10.3	75	23.70	13.2	101	7.8	0.077	23.70	1.81	21.89
113-119	10.3	10.8	76	24.95	25.7	112	16.3	0.146	24.95	3.62	21.33
133-139	10.3	12.2	84	30.00	52.8	109	27.9	0.256	30.00	7.70	22.30
139-144	9.2	12.5	88	31.75	54.4	100	24.2	0.242	31.75	7.70	24.05

elevated; at higher urine flow of 10 cc. per minute still higher clearances were observed.

The ratio amino acid clearance/creatinine clearance remained constant throughout the control periods but increased as the plasma amino acid N concentration rose and was accompanied by greater excretion of filtered amino acid. Fluctuations in glomerular filtration during the infusion of dl-alanine were like those noted in the control periods and at no time was glomerular filtration increased by the injection of the material.

Similar studies were carried out with casein hydrolysate (table 3—expts. 10, 11 and 12). Using different dilutions of the 10 per cent casein hydrolysate solution, plasma amino acid N levels ranging from 11 to 41 mgm. per 100 cc. were attained. As with dl-alanine the amounts of amino acid excreted paralleled the increase in plasma value resulting in high renal amino acid clearances; similarly, with the higher rates of urine flow higher clearances were obtained. The ratio amino acid clearance/creatinine clearance increased in direct proportion to the plasma concentration. In experiments 11 and 12 urea clearances were obtained. The ratio urea clearance/creatinine clearance was constant throughout the control periods, as well as during the infusions of casein hydrolysate.

A maximal rate of tubular transfer for alpha-amino acids could not be demon-

TABLE 3

Results of continuous intravenous infusions of casein hydrolysate in normal dogs

TIME	URINE FLOW	MG./100 CC.						MAXIMUM CLEARANCE (CC./MIN./SQ.M.)			RATIO CLEARANCES		AMINO ACID N (MG./100 CC. OF GLOMERULAR FILTRATE/SQ.M.)			
		Creatinine		Amino Acid N		Urea N		Creatinine	Amino Acid	Urea	A.A. CREAT.	UREA CREAT.	Filtered	Excreted	Reabsorbed	
		Plasma	Urine	Plasma	Urine	Plasma	Urine									
Experiment 10																
minutes	cc./min.															
0	Gavage with 600 cc. water and 0.33% creatinine to dog 963 (15.9 kilo, S.A. = 0.6846)															
20	Continuous I.V. with 2.5% casein hydrolysate† (0.17% NH ₂ N) 0.25% creatinine at 10 cc. per minute															
35-43	4.3	15.1	246	10.89	2.0			101	1.2		0.012		10.89	0.12	10.77	
43-54	5.8	16.4	248	10.89	4.8			127	3.7		0.029		10.89	0.32	10.57	
54-63	5.2	17.8	246					105								
64	Continuous I.V. with 5% casein hydrolysate‡ (0.34% NH ₂ N) 0.25% creatinine at 10 cc. per minute															
76-85	4.9	20.6	370	16.82	11.5			128	4.9		0.038		16.82	0.64	16.18	
85-95	4.7	21.6	350	16.82	17.3			111	7.1		0.064		16.82	1.08	15.74	
95-100	6.2	22.3	320					130								
101	Continuous I.V. with 7.5% casein hydrolysate‡ (0.51% NH ₂ N) 0.25% creatinine at 10 cc. per minute															
110-120*	12.0	26.5	201	40.75	71.4			133	30.7		0.231		40.75	9.41	31.34	
120-128*	14.0	28.5	154	40.75	78.8			110	39.5		0.359		40.75	14.65	26.10	
Experiment 11																
0	Gavage with 400 cc. water to dog 395 (13.1 kilo, S.A. = 0.5894)															
32	Continuous I.V. with 0.25% creatinine in 5% glucose in saline at 10 cc. per minute															
73-88	1.8	10.6	352	3.00	0.4	5.34	104	103	0.4	60.5	0.004	0.59	3.00	0.01	2.99	
88-103	2.1	11.1	400	2.80	1.2	5.19	110	127	1.5	74.2	0.012	0.59	2.80	0.03	2.77	
110	Continuous I.V. with 5% casein hydrolysate§ (0.34% NH ₂ N) 0.25% creatinine at 9 cc. per minute															
130-145	2.1	15.0	600	8.95	24.7	5.95	181	140	9.7	106.6	0.069	0.76	8.95	0.62	8.33	
145-155	3.5	16.1	578	10.00	29.4	7.20	104	213	17.5	85.7	0.082	0.40	10.00	0.82	9.18	
Experiment 12																
0	Gavage with 400 cc. water to dog 395 (13.1 kilo, S.A. = 0.5894)															
45	Continuous I.V. 5% glucose in saline at 8 cc. per minute															
53	Continuous I.V. with 10% casein hydrolysate§ (0.68% NH ₂ N) at 8 cc. per minute															
58-73	1.4	13.0	530					97								
73-88	1.9	18.3	661	17.70	60.4	14.00	300	115	10.8	68.0	0.094	0.59	17.70	1.66	16.04	
88-98	5.0	25.8	330	24.70	83.3	15.90	114	108	28.5	60.6	0.264	0.56	24.70	6.55	18.15	
98-108	4.2	27.2	397	26.50	59.1	17.50	152	104	15.9	62.0	0.153	0.60	26.50	4.04	22.46	
108-114†	5.2	33.5	323	33.50	59.2	19.90	119	85	15.5	52.5	0.183	0.62	33.50	6.12	27.38	

* Vomited.

† Infusion rate 15.5 cc./min.

‡ pH 4.58.

§ pH 5.60.

strated at the plasma amino acid concentrations reached, about tenfold the fasting level, with either dl-alanine or casein hydrolysate (fig. 1). An unexpected finding was the similarity of the pattern of tubular reabsorption of such different substances as dl-alanine and casein hydrolysate. Progressive increase in the amount of amino acid excreted paralleled the rise in plasma amino acid N concentration, and at high plasma amino acid N levels (30-40 mgm./100 cc.) reabsorption varied from 64 to 81 per cent. No evidence of active tubular excretion was noted. The ability of the renal tubules to reabsorb alpha-amino acids from the glomerular filtrate was remarkably efficient.

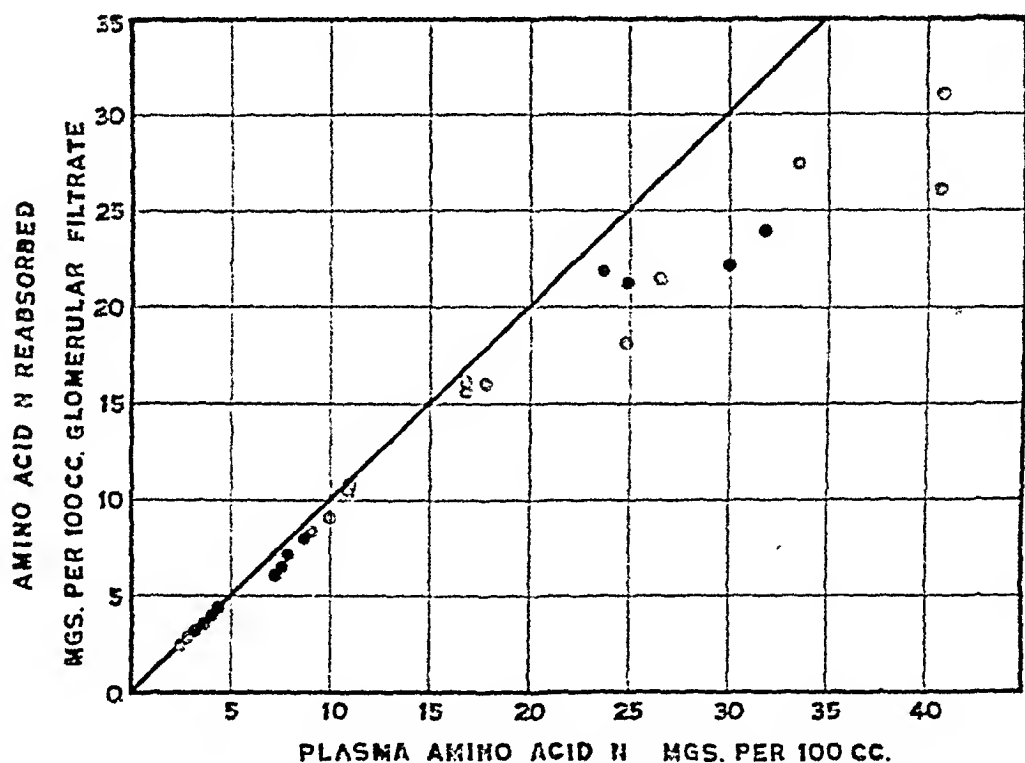


Fig. 1. Relation between plasma amino acid N concentration and amounts of amino acid N reabsorbed from the glomerular filtrate (per 100 cc. per sq.m.). The diagonal line represents complete tubular reabsorption from the glomerular filtrate; the solid dots data obtained during the infusion of dl-alanine and the open circles during the infusion of casein hydrolysate.

COMMENT. Shannon (1942) recently reviewed the literature concerning renal tubular transfer. With regard to glucose (Shannon and Fischer, 1938) and ascorbic acid (Ralli et al., 1940) adequate studies have demonstrated that a maximal rate of tubular reabsorption exists. The question of a maximal rate of tubular reabsorption of inorganic phosphate has not been settled (Harrison and Harrison, 1941; Ollayos and Winkler, 1943). The experiments reported here fail to demonstrate a maximal rate of reabsorption for alpha-amino acids with plasma levels up to ten times the normal fasting value. The limited data presented suggest, however, that the maximal rate was approached. It should be appre-

ciated that although the test materials were dl-alanine, glycine and casein hydrolysate, the substance measured in the blood and urine was alpha-amino acid N. Hence the clearances should be thought of as alpha-amino acid clearances.

Tubular reabsorption of active metabolites can be measured reliably only if the plasma concentration of the substance studied does not vary significantly during the period of observation. Since the avidity of the body tissues for amino acids results in very rapid alteration of plasma concentration it may be questioned whether amino acid clearances determined in the conventional fashion are valid. The reliability of the data for reabsorption is dependent on the speed with which equilibrium is reached between the tubular cells and the glomerular filtrate. In our experiments short urine collection periods were selected in an attempt to minimize the effect of the unavoidable rapid alterations in plasma concentration.

Interpretation is further clouded by the rapid chemical interchange within the body. As Schoenheimer and his co-workers have shown (1942), amino acids may undergo immediate chemical transformations, such as deamination, transamination, and reamination, which allow rapid exchange of ingested or infused amino acids with the body pool. If rapid transamination occurs it is evident that alpha-amino acid N measured in plasma and urine even at short intervals after injection of dl-alanine need not truly reflect the concentration of dl-alanine. Immediate chemical transformation would seem to offer an adequate explanation for the similarity of tubular reabsorption observed after administration of such different substances as dl-alanine and casein hydrolysate.

SUMMARY

The urinary excretion and reabsorption of alpha-amino acid following oral and intravenous administration of dl-alanine and of casein hydrolysate, with plasma levels of alpha-amino acid N up to ten times the normal value, follow a uniform pattern in the normal dog. At normal plasma amino acid N concentration the renal amino acid clearance was usually less than 1 cc. per sq.m. per min. Progressive increase in the amount of amino acids excreted in the urine paralleled the rise in plasma amino acid N concentration with resultant high amino acid clearances. The efficiency of the renal tubules in reabsorption of amino acids from the glomerular filtrate was remarkable; even at very high plasma amino acid N concentrations as much as 64 to 81 per cent was reabsorbed. A maximal rate of tubular transfer was not demonstrated for either substance at the plasma concentrations attained, and there was no evidence of tubular excretion of amino acids.

The ratio urea clearance/creatinine clearance remained fixed during the progressive increase in both plasma amino acid and urea concentrations. In contrast the ratio amino acid clearance/creatinine clearance increased with the rise in plasma amino acid.

We wish to express our appreciation to Dr. James A. Shannon for his helpful criticism and advice.

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THE EFFECT OF THYROXIN ON THE MAXIMUM RATE OF TRANSFER OF GLUCOSE AND DIODRAST BY THE RENAL TUBULES

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The purpose of the present investigation was to study the effect of thyroid hormone on the maximum rate of absorption of glucose by the renal tubules of the dog. Our interest in this problem goes back to the observation (1) that the hyperthyroid state in the rat induces, apparently in a specific manner, a marked increase in the rate of absorption by the intestine of glucose and of other substances susceptible to phosphorylation. We were interested to learn whether or not the thyroid hormone has the same effect on the rate of absorption of glucose by the renal tubules. Also, it might be pointed out that when these studies were initiated little had been published concerning the endocrine control of the tubular transfer of organic solutes. Since that time several papers have appeared that deal with this phase of renal physiology (2) (3) (4).

Early in the work when it seemed certain that thyroxin did accelerate the maximum rate of the tubular absorption of glucose, it became apparent that a study of the effect of thyroxin on the rate of the tubular transfer of a second substance showing a maximum rate of transfer might help to shed light on the mechanism whereby thyroxin exerts its effect. To these ends we have studied the influence of thyroid hormone on the maximum rate of transfer of diodrast (DT_m) and of glucose (GT_m).

METHODS. The maximum rate of the tubular transfer of glucose was determined by the procedure outlined by Shannon and Fisher (5). The creatinine clearance was employed as a measure of the rate of glomerular filtration. The maximum rate of transfer was determined as the difference between the amount of glucose filtered through the glomeruli each minute and the amount of glucose excreted during the same period.

Three well trained unanesthetized female dogs weighing between 15 and 17 kilos were used. Adequate diuresis was obtained through the administration of 40 cc. of water per kilo of body weight by stomach tube about 1 hour prior to the start of the initial urine collection period. Desirable concentrations of glucose and of creatinine in the plasma were obtained by a priming intravenous injection of a solution of these substance in physiological salt solution. The plasma concentrations were maintained at a constant level throughout the experimental period by a constant intravenous injection of a second solution of these chemicals.² When it was found desirable to determine the maximum rate for the tubu-

¹ Aided by a grant from the Christine Breon Fund for Medical Research.

² A relatively inexpensive and very serviceable pump for this purpose was constructed by the Industrial Instrument Company, 116 New Montgomery Street, San Francisco, California.

lar excretion of diodrast, this substance in calculated amounts, was added to both the priming and the constantly infused solutions. An arterial blood sample was obtained for each urine collection period. All blood samples were obtained approximately 2 minutes before the mid-point of the urine collection period. In this fashion (4) one may correct for the effect of any changes in the plasma concentrations of glucose, creatinine, or diodrast. However, little difficulty was experienced in maintaining the plasma concentrations at levels that were constant within the limits reported by Shannon and co-workers (4) (5). The concentration of creatinine was maintained at plasma levels between 25 and 40 mgm. per cent. The plasma glucose was maintained at such a level that the ratio of the amount of glucose filtered through the glomeruli to the amount of glucose absorbed by the renal tubules lay between 1.5 and 2.5. In general, the plasma glucose was maintained constant at levels that ranged between 200 and 600 mgm. per cent. The concentration of diodrast was maintained near the 20 mgm. per cent level. Urine was collected by catheter and the last traces were removed by washing the bladder with 100 cc. of physiological salt solution at body temperature. Each collection period extended through an exactly timed period of about twenty minutes. The first period was started between 30 and 40 minutes after beginning the constant intravenous infusion.

Creatinine was determined on tungstic acid filtrates of plasma and on untreated, diluted urine by the method of Folin and Wu (1919). Interference due to the presence of glucose was avoided by following the precautions observed by Shannon and Fisher (5). Plasma glucose was determined as the difference between the reducing value of a tungstic acid filtrate before and after fermentation by yeast according to the method of Hagedorn and Jensen (6). Urinary glucose was determined on a filtrate prepared according to West and Peterson (7). The Hanes (8) modification of the Hagedorn and Jensen method was used to determine the reducing value before and after treatment with yeast. Diodrast was determined on tungstic acid filtrates of plasma and on diluted samples of urine according to the method of Alpert (9). Tests were conducted to show that the presence of glucose and creatinine did not interfere with the accuracy of the determination of diodrast. Diodrast T_m was calculated as the difference between the amount of diodrast excreted per minute and the product of the creatinine clearance and the plasma concentration of filterable diodrast. The latter value was calculated from the total plasma diodrast according to the method and figures of Smith and Smith (10). In order to make these calculations it is necessary to know the concentrations of plasma albumin and total plasma proteins. These were determined according to the method of Kraus (11).

The experiments were planned so that each animal served as its own control. First, several series of determinations were made on the animal in the normal state. The animal was then rendered hyperthyroid through the daily feeding of large doses of Armour's desiccated thyroid substance or through the subcutaneous injection of synthetic *dl*-thyroxin. Dosage and duration of administration are reported in table 1. When an increase in pulse rate and rectal temperature was accompanied by diuresis and a loss of weight as indications of a hyperthyroid state, a second series of determinations was made.

TABLE 1

Creatinine clearance, glucose Tm, diodrast Tm, and rectal temperature in normal and hyperthyroid dogs

DOG	DATE	STATE OF ANIMAL	NUMBER OF PERIODS	CREATININE CLEARANCE	GLUCOSE Tm	DIODRAST Tm	RECTAL TEMPERATURE	LOSS OF WEIGHT DURING THYROID REGIME	DOSE OF THYROID HORMONE	NUMBER OF DAILY DOSES
				cc./min.	mgm./min.	mgm. I ₂ /min.	°C.	Kilo		
A	10/10/40	Control	2	61	187		39.1			
	10/24/40	Control	2	59	202		39.2			
	1/29/41	Control*	3	66	195		38.6			
	Average			63±4†	195±10†		39.0			
	11/ 9/40	Hyperthyroid	3	69	211		39.6		5 grams thyroid substance	5
	11/19/40	Hyperthyroid	3	74	224		39.6	1.4	5 grams thyroid substance	15
	11/28/40	Hyperthyroid*	3	65	161		39.5	1.4	5 grams thyroid substance	24
	2/24/41	Hyperthyroid*	3	74	191		39.6		6.4 grams thyroid substance	21
	Average			70±4	197±27		39.6			
	9/24/41	Hyperthyroid	2	72	286		39.6	1.0	20 mgm. Thyroxin	8
	9/29/41	Hyperthyroid	5	77	287		39.5	1.6	20 mgm. Thyroxin	12
	10/10/41	Hyperthyroid*	4	76	249		39.3	1.8	20 mgm. Thyroxin	14
	Average			70±2	273±23		39.4			
	2/12/41	Control	3	54	186		38.2			
	8/12/41	Control	3	54	168		38.0		4 months after termination of thyroid feeding	
	8/15/41	Control	3	48	182		38.1			
	10/ 8/41	Control*	4	65	190		38.2		1 month after termination of thyroid feeding	
	2/ 3/42	Control	2	46	149	7.4	38.3		3 months after termination of thyroxin regime	
	2/12/42	Control	3	53	167	8.8	38.5			
	2/19/42	Control	3	40		10.1	38.3			
	2/26/42	Control	3	48	163	8.8	38.6			
	Average			52±7	174±16	8.9±1	38.3			
B	3/27/41	Hyperthyroid	3	69	235		39.2		6 grams thyroid substance	26
	4/ 3/41	Hyperthyroid*	3	66	176		39.3	1.4	7 grams thyroid substance	34
	4/ 7/41	Hyperthyroid	2	62	213		39.0		7 grams thyroid substance	38
	Average			66±4	207±29		39.2			
	9/10/41	Hyperthyroid*	5	65	217		38.9	1.2	10 mgm. Thyroxin	10
									20 mgm. Thyroxin	7
	10/22/41	Hyperthyroid*	6	74	209		39.2	1.8	40 mgm. Thyroxin	7
	10/27/41	Hyperthyroid	4	76	265		39.2	2.6	40 mgm. Thyroxin	11
	10/29/41	Hyperthyroid	3	79	211		39.4	2.6	40 mgm. Thyroxin	11
									20 mgm. Thyroxin	2
	3/19/42	Hyperthyroid	3	76	230	19.1	39.1	1.6	45 mgm. Thyroxin	6
	3/26/42	Hyperthyroid	3	76	212	23.1	39.3	2.5	45 mgm. Thyroxin	10
	3/31/42	Hyperthyroid	3	74	226	15.0	39.3	2.6	45 mgm. Thyroxin	13
	Average			74±5	224±20	19.1±3	39.2			
	4/23/42	Control	3	63		13.5	38.5			
	4/28/42	Control	3	63	209	11.2	38.4			
	4/30/42	Control	3	63	218	12.3	38.6			
	Average			63±2	214±13	12.4±1	38.5			
	5/22/42	Hyperthyroid	3	90	311	16.1	39.0		40 mgm. Thyroxin	5
	5/25/42	Hyperthyroid	3	92	358	20.5	39.3	0.4	40 mgm. Thyroxin	8
	Average			91±3	335±25	18.3±2	39.2			

* In these experiments the perfusion fluids contained a sufficient amount of neutralized succinic acid so that the animals received 120 mgm. succinic acid per minute.

† Mean values and standard deviations.

The rectal temperature of both the normal and hyperthyroid animal was observed closely and is reported in the data. The animal in the hyperthyroid state received the same amount of a Cowgill's diet as in the normal condition. During the hyperthyroid period the diet was fortified with extra amounts of thiamin. To avoid lowering the body temperature all fluids were administered at a temperature of about 37°.

RESULTS AND DISCUSSION. The results are presented in table 1. In order to conserve space only the average of the data for the several periods of a given experiment is given. The number of periods in a given experiment is indicated.

The data for the animals in the normal state stand in good agreement with that of previous workers (2) (4) (5), and confirm the observation (4) that the mechanism involved in the transfer of glucose is quite stable.

The data for the thyroxinized animals show quite clearly that the administration of thyroxin leads to a definite increase in the creatinine clearance, the glucose T_m , and the diodrast T_m . The feeding of thyroid substance did not have as marked an effect on the glucose T_m as did the administration of thyroxin al-

TABLE 2
Comparison of data for normal and hyperthyroid animals

DOG		INCREASE OVER NORMAL IN PER CENT			INCREASE IN RECTAL TEMPERATURE
		Creatinine clearance	GT_m	DT_m	
A	Thyroid substance	11	0		+ .6
	Thyroxin	21	40		+ .4
B	Thyroid substance	27	19		+ .9
	Thyroxin	42	29	115	+ .9
C	Thyroxin	45	56	48	+ .7

though the clinical signs of hyperthyroidism and the rise in rectal temperature were about the same under both types of therapy. The results relative to the influence of thyroid hormone on the diodrast T_m confirm the work of Heinbecker, Rolf and White (12) which appeared during the preparation of this manuscript. In table 2 the data for the animals in the hyperthyroid state are given in terms of the per cent increase over the data for the normal animals.

Since the data relative to the changes in the rate of glomerular filtration, as measured by the creatinine clearance, may be explained on the basis of an altered glomerular dynamics (13), our attention will be confined to the changes in the maximum rates for the tubular transfer of glucose and diodrast. Increases in the rate of flow of blood have little or no effect on the magnitude of the glucose T_m and the diodrast T_m (14).

The observations relative to the effect of thyroxin, and to a lesser extent of thyroid substance, on the rates of maximum tubular transfer stand in relation to the work of White, Heinbecker and Rolf (2) in the hypophysectomized dog. These investigators have shown that within seven days after hypophysectomy,

the diodrast T_m may fall to 50 per cent of the normal value. The diminution of diodrast T_m is accompanied by a decrease in the renal blood flow. A further drop in the rate of the tubular excretion of diodrast is observed subsequent to the initial drop. Due to the early onset of the effect of hypophysectomy, the authors suggest that the changes that were noted are to be attributed to a direct effect of the removal of the hypophysis and not to a secondary change in other endocrine glands. Although a large share of the immediate drop in the diodrast T_m may be explained by a diminished renal blood flow, the demonstrated effects of thyroxin on the maximum rates for the transfer of both glucose and diodrast suggests that the subsequent fall in the tubular excretion of diodrast effected by hypophysectomy may be attributed to a decrease in thyroid activity. In this connection, it has been demonstrated that the subnormal rate for the intestinal absorption of glucose shown by the hypophysectomized rat can be restored to normal through the administration of very small doses of thyroxin (15). Heinbecker, Rolf and White (12) have shown that both thyroid extract and a hypophyseal extract produce a marked increase in the diodrast T_m in the normal and in the hypophysectomized dogs. In the light of these observations it would be interesting to learn the effect of hypophyseal extracts on the thyroidectomized dog.

A question arises concerning the mode of the action of thyroid hormone on the maximal rates of tubular transfer. The following factors are conspicuous among those that may be involved: *a*, opening of previously inactive nephrons; *b*, hypertrophy of the existing renal tubules; *c*, increase in functional activity of tubular tissue due to a rise in temperature, and *d*, a catalytic effect of thyroid hormone on the cellular mechanisms involved in the transfer processes. In view of the fact that the maximal rates for the tubular transfer of glucose and diodrast are employed as measures of the number of open glomeruli and of the amount of active tubular tissue (14), it is important to attempt to ascertain whether the effect of thyroxin is due to an increase in the effective tubular mass (*a* and/or *b*) or to an increase in the functional capacity of a given tubular mass (*c* and/or *d*).

It is highly improbable that the effects of thyroxin are to be attributed to the opening of previously inoperative nephronic units. It has been shown from histological studies that all of the glomeruli in the kidneys of the normal dog (16), as well as of the hypophysectomized dog (2) are functioning continuously. The stability of the glucose T_m and the diodrast T_m in the normal animal during experimental procedures involving marked changes in the rates of glomerular filtration and renal blood flow also speaks against the inactivity of a significant number of nephrons (4) (14).

It is now well established that several of the products of the endocrine glands exert a renotropic effect (17). According to Swann (18), the administration of thyroxin to rats for a period of a month may lead to a 35 per cent increase in the weight of the kidneys. The work of MacKay and MacKay (19) suggests that even greater increases in kidney weight may be expected from thyroid feeding. Inasmuch as the data indicating an increase in the size of the kidney due to thyroid therapy are reported in terms of wet weight, it is not known to what extent the increase in weight may be attributed to an increase in active tubular mass and to what extent it may represent an accumulation of fluid. In any event it

would seem problematical whether or not a renotropic effect from thyroid hormone can be considered apart from an increase in the functional activity of the tubular mass. The data of Addis (20) strongly suggest "that the stimulus to growth of the nephron may lie in the relation between the oxygen consumption required for work and the cell mass engaged in work." Belasco (21) has demonstrated that an increase in the functional activity of the kidney, measured by oxygen consumption, accompanies the increase in wet weight induced by thyroid feeding. It is presumed that any hypertrophy that may have occurred in our animals is a structural adaptation to the increase in activity occasioned by thyroid hormone.

According to Shannon (22), the important characteristics of a transfer process may be accounted for if we assume that the solute in question enters into a reversible and transitory combination with a cellular constituent that is present in limited amount. The rate of a transfer process involving such a sequence of chemical reactions is expected to be dependent upon several variables. Studies (23) conducted on the human being indicate that a rise in rectal temperature of one degree Fahrenheit increases the rate of the tubular transfer of glucose 10 per cent. According to these data, and using rectal temperature as a criterion of the change in body temperature, the small changes in the rectal temperatures observed in the hyperthyroid animals are not sufficient to account for a significant share of the increase in the maximal rates of transfer of glucose and diodrast. As is seen in table 2, and considering only the animals treated with thyroxin, the greatest increase in rectal temperature (0.9°) was not sufficient to account for more than a 15 per cent increase in the rates of transfer, yet the values for the glucose T_m increased between 29 and 56 per cent and the values for the diodrast T_m increased 48 and 115 per cent. It should be borne in mind, however, that the changes in the temperature of the kidney may exceed those of the rectum (24). However, the data for the animals treated with thyroid substance militate against the possibility that an increase in the temperature of the kidney may have accounted for the increase in glucose T_m and diodrast T_m . Although the animals treated with thyroid substance showed the same rise in rectal temperature (and presumably, in kidney temperature) as the animals treated with thyroxin, the change in the rate of transfer of glucose was not nearly so marked.

While an increase in the temperature of the kidney, together with a functional hypertrophy,³ may be sufficient to account for the increases that were observed, a change in the rates of the transfer processes (glucose and diodrast) due to a direct effect of thyroid hormone on the transfer mechanism is supported by previous work. Studies (1) conducted on the rate of absorption of various substances by the intestine showed that administration of thyroxin increased, and thyroidectomy decreased, the rates only in the case of those substances showing an active absorption by a mechanism generally considered to involve phosphorylation. An attempt was made to exclude, as causative factors, such variables as the emptying time of the stomach, temperature, rate of blood flow,

³ The observation made in this laboratory that thyroxin does not increase the rate of the tubular absorption of galactose, a substance which is absorbed passively, does not speak favorably for a marked hypertrophy of the renal tubules.

peristaltic activity, etc. It was concluded that thyroxin influences the rate of absorption of substances susceptible to phosphorylation.

Although it is unlikely that the transfer of diodrast is mediated by the same mechanism that is involved in the transfer of glucose, it is reasonable to suppose that any effect of thyroxin accounting for an increase in the activity of the mechanism involved in the transfer of glucose should account, also, for an increase in the transfer of diodrast. According to the hypothesis advanced by Wilbrandt and Laszt (25), and employed by Lundsgaard (26) and others, the active absorption of glucose is dependent upon its conversion to hexosephosphate in the epithelial cells (27). In this manner there is obtained a steep gradient for the diffusion of the sugar across the membranous barrier proximal to the lumen. Hydrolysis of the ester must occur before the sugar reaches the circulation. Recent evidence (for reviews see (13) (28) (29)) supports the view that glucose is phosphorylated by the epithelial cells of the intestine and the proximal renal tubules. Although phlorhizin inhibits the transfer in the renal tubules of diodrast (30), as well as of glucose (26), it is improbable that obligate phosphorylation is involved in the transfer of this molecule. The portion of the molecule susceptible to phosphorylation is attached to the iodine containing moiety only through an electrostatic linkage. The substances that are considered (31) to compete with diodrast for a cell constituent involved in the transfer mechanism are not substances that are known to be phosphorylated by tubular tissue, such as glucose, but are substances (phenol red and hippuran) that are equally unlikely to be phosphorylated. It has not been demonstrated, however, that phosphate, in the sense of the hypothesis of Shannon, is the cell constituent involved in the transfer of glucose, and that obligate phosphorylation is concerned in the transfer of glucose. Verzar and Sullmann (32) and others have suggested that the increase in the various acid-soluble organic phosphate fractions in the mucosal epithelium that accompanies the absorption of glucose may represent only an adventitious phosphorylation occasioned by an increase in cell activity and the presence of a plethora of substrate. Lipmann (29) has pointed out that a cellular mechanism of transfer dependent upon a change in diffusion gradient due to obligate phosphorylation would represent a poor utilization of phosphate bond energy. Also, the localization of phosphatase in the epithelial cells of the proximal tubules (29) is not such as to inspire confidence in such a mechanism (33).

The data relative to the effects of thyroxin on the diodrast T_m and glucose T_m may be brought into accord, however, if the rôle of phosphorylation as a transmitter of oxidative energy (28) (29) (34) is stressed, and no attempt is made to define the specific mechanisms (glucose and diodrast) whereby this energy is utilized. Such a view does not exclude the possibility of the involvement of obligate phosphorylation in some phase of the transfer of glucose; it does, however, allow for a more general use of the energy transmitted by way of high energy phosphate bonds. The effects of phlorhizin (35) (36) are equally well explained.

Kalckar (35), Colowick, Kalckar and Cori (37), and Ochoa (38) have shown that the phosphorylation that occurs in homogenized kidney and other tissues

is coupled with respiration. In these preparations the energy made available through the oxidation of suitable substrates is transferred, via the adenylic acid system, to glucose and other substances susceptible to phosphorylation as phosphate bond energy. Undoubtedly the transfer of oxidation energy is made to better purpose in the organized cell, i.e., such energy might be transferred to the mechanisms involved in the active transfer of solutes. According to these data it is possible that the well known effect of thyroxin on respiration can account for an increase in the rate of transfer of energy by way of the adenylic system to the mechanisms involved in the transfer of glucose, diodrast, and other substances. It is attractive to consider that the thyroid hormone exerts its effects upon some part of the system involved in the transfer of phosphate energy and in this way exerts its characteristic control over the rate of oxygen consumption.

It is proposed that thyroid hormone influences the activity or availability of one of the factors involved in the transfer of phosphate bond energy. Data are available that exclude the possibility of a change in the status of two of these factors. Earlier studies (33) carried out in this laboratory on the intestinal mucosa demonstrated that the state of the activity of the thyroid does not influence the concentration of adenosinetriphosphate. The concentration of the 7 minute hydrolyzable phosphate in the mucosa during the absorption of glucose, is the same in the normal, the hyperthyroid, and the thyroidectomized animal. The data shown in table 1 relative to the influence of succinic acid on the glucose T_m indicate that a deficiency of an essential, easily oxidized substrate (39) cannot account for the limitation of the maximum rate of transfer in the normal animal. The addition of succinic acid to the perfusion fluid did not alter the magnitude of the glucose T_m in the normal animal. The effect of succinic acid on the tubular transfer of the animal in the hyperthyroid state was toward a decrease in glucose T_m .

The possibility of the involvement of a third factor is rendered probable by the recent studies of Bailey (40) and Dubois and Potter (41). The work of these investigators lends support to the rôle of adenosine-triphosphatase as a catalyst in the transfer of phosphate bond energy. Changes in the concentration or activity of this enzyme, due to an influence of thyroid hormone, could account for an increase in the rate of transfer of energy and in the rate of utilization of oxygen. This possibility is being studied at the present time.

SUMMARY

1. A study was made of the effect of thyroid therapy on the rates of transfer of glucose and diodrast by the renal tubules of the dog.
2. Administration of thyroxin led to a marked increase in the creatinine clearance, the glucose T_m , and the diodrast T_m .
3. The possible causes of the effect of thyroxin are discussed.
4. It is suggested that thyroxin exerts its effect on the maximum rates for the transfer of glucose and diodrast, as well as its characteristic effect on respiration through activation of the system involved in the transfer of phosphate energy, possibly through activation of adenosinetriphosphatase.

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STIMULATION OF PEPSIN SECRETION BY MEANS OF ACID IN THE INTESTINE¹

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Abundant experimental evidence has accumulated which leads to the conclusion that a humoral mechanism is involved in the pyloric (or gastric) phase of gastric secretion. However, although the gastric juice of the gastric phase normally has a moderate peptic activity, the juice secreted in response to gastrin or histamine, the supposed gastric hormones, is very low in peptic power.

A possible explanation for the difference in composition between "pyloric phase" juice and "gastrin" juice is that in addition to the known humoral principle which is apparently specific for the parietal cells, there may exist another humoral principle which is specific for the chief cells. Since the pyloric and intestinal phases of gastric secretion generally overlap, much of the pepsin of the pyloric phase may be the result of stimuli arising in the intestine. The experiments of Pratt (1940) and Babkin and Komarov (1941) suggest that the small intestine may be the site of elaboration of a hormone having pepsigogue effect. Pratt reported that the administration of Agren's secretin resulted in the secretion of pepsin. Babkin and Komarov (1941) and Bucher and Greengard (1942), using their own preparations of highly purified and crystalline secretin, could not confirm Pratt's results. Babkin and Komarov, however, were able to prepare extracts of duodenal mucosa which had a low pancreatic but a pronounced pepsigogue effect and concluded that the secretin used by Pratt probably was contaminated by an intestinal pepsigogue.

In the present experiments we attempted to determine whether a pepsigogue effect could be elicited by means of acid instilled into the small intestine. Acid was chosen as the stimulus since it normally enters the intestine during the course of gastric secretion. In addition, other "digestive hormones" of the small intestine (secretin, cholecystokinin, enterocrinin, villikinin) are said to be elaborated following the instillation of acid.

METHODS. Three dogs equipped with a Pavlov pouch of the stomach and one with a Heidenhain pouch were used. The Pavlov pouches were prepared by the technique described elsewhere (Thomas, 1942). In addition to the gastric pouch, each dog was also provided with cannulated gastric and duodenal fistulas (Thomas, 1941). Acid was instilled continuously at a constant rate into the bulb of the duodenum by means of a rubber tube passed through the duodenal cannula. Samples of duodenal contents for pH determinations were taken every half hour from a point approximately 15 cm. distal to the entrance of the acid. Determinations of pH were made electrometrically, using a glass electrode.

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Peptic activity was measured by a modification of the Mett method which we have found to give accurate and reproducible results (Friedman and Bennett, 1943). Acidities of the gastric secretion were determined in the usual manner.

The animals were all in good health and kept on a standard diet of ground beef, Purina chow, bread, milk, and acid. The acid was introduced with the food in order to replace that lost daily from the draining gastric pouch; the volume of 0.1 N HCl so fed was approximately equal to the gastric juice lost daily. Experiments were performed 18 to 24 hours after the last meal and only when a control period of 1 to 2 hours revealed that the stomach was in a fasting state.

The acid introduced into the intestine was of varying concentrations, ranging from 0.1 N to 0.15 N. In a few experiments blood appeared in the intestinal contents during acid instillation; such experiments were discarded. Control experiments were performed during which 0.9 per cent NaCl was infused into the small intestine instead of acid.

RESULTS. In the fasting state, gastric secretion from the pouch averaged about 3.2 cc. per hour. The secretion consisted chiefly of mucus, the liquid portion having a pH of 1.61 to 3.52, a total acidity of about 50 m. eq./l. and a pepsin concentration of about 208 units. Acid instillation did not affect the acidity of the fasting secretion but apparently did increase the concentration and total output of pepsin, but usually only after the intestinal instillation of acid was stopped. In one dog the average total output of pepsin was increased 27 per cent and in another 65 per cent. No consistent effect on the pepsin output was found when 0.9 per cent NaCl was substituted for the acid.

The small volume of the fasting secretion and the relatively large proportion of mucus made pepsin determinations difficult and the results inconclusive. We therefore studied the effect of superimposing the intestinal stimulus (acid) on some gastric stimulus (feeding, insulin, or histamine). After determining the level of pepsin secretion in response to a test meal, insulin, or histamine, the experiment was repeated with the addition of infusing acid into the intestine. Since some of us (Pincus, Thomas and Reh fuss, 1942) had found previously that the volume and concentration of acid secreted by the stomach was greatly reduced when the intestinal contents reached a pH of about 2.5 or lower, careful determinations of intestinal pH were made to note if a similar intestinal pH threshold existed with respect to pepsin secretion.

Only seven test-meal experiments were performed on the Heidenhain pouch dog because a round worm infection developed later and made her unfit for further use.

Influence on pepsin secretion in response to a test meal. The standard test meal consisted of 300 grams ground beef heart free from visible fat. Various time intervals between the feeding and the beginning of the acid infusion were tried, ranging from a few minutes to two hours. The infusion of acid into the intestine was maintained for periods ranging from 30 minutes (when 60 cc. of acid were used) to 2½ hours (when 560 to 740 cc. of acid were used).

A series of representative experiments on Pavlov-pouch dogs is summarized in tables 1 and 2. During the period of acid instillation the total output of

TABLE 1

Effect of instilling acid into the small intestine on gastric secretion in response to a meat meal—Dog "D"

NUMBER OF EXPERIMENTS	ACID INSTILLED INTO INTESTINE IMMEDIATELY FOLLOWING MEAL OF 300 GRAMS BEEF			HOUR														
				1			2			3			4			5		
				Volume	Pepsin		Volume	Pepsin		Volume	Pepsin		Volume	Pepsin		Volume	Pepsin	
					Concentration	Output		Concentration	Output		Concentration	Output		Concentration	Output		Concentration	Output
6	Control, food only			cc.	units	units	cc.	units	units	cc.	units	units	cc.	units	units	cc.	units	units
				52.6	127	10,490	50.0	124	9,920	53.1	99	5,235	30.0	113	3,390	21.4	145	3,103
4	700 cc. 0.10 N	2 hr.	>2.5	Per cent of control			Per cent of control			Per cent of control			Per cent of control			Per cent of control		
				79	194	153	109	173	173	144	164	230	205	142	292	231	109	231
3	760 cc. 0.10 N	2 hr.	<2.3	26	209	49	17	265	47	77	144	113	246	81	202	295	72	210

TABLE 2

Effects of instilling acid and saline into the small intestine on gastric secretion in response to meat meal—Dog "B"

NUMBER OF EXPERIMENTS	FLUID INSTILLED INTO INTESTINE IMMEDIATELY FOLLOWING MEAL OF 300 GRAMS BEEF			HOUR														
				1			2			3			4			5		
				Volume	Pepsin		Volume	Pepsin		Volume	Pepsin		Volume	Pepsin		Volume	Pepsin	
					Concentration	Output		Concentration	Output		Concentration	Output		Concentration	Output		Concentration	Output
11	Control, food only	cc.		cc.	units	units	cc.	units	units	cc.	units	units	cc.	units	units	cc.	units	units
				65.4	120	7,855	67.2	99	6,653	49.9	72	3,534	33.8	47	1,558	27.2	44	1,200
4	0.15N HCl	740	2 hr. 45 min.	Per cent of control			Per cent of control			Per cent of control			Per cent of control			Per cent of control		
				42	113	47	55	172	95	74	236	182	150	238	355	199	168	329
6	0.15N HCl	60	30 min.	<2.5	95	92	85	100	90	93	107	100	103	119	110	122	97	111
4	0.9% NaCl	540	2 hr.	>3.3	92	108	99	110	96	105	129	83	107	154	77	211		

pepsin was increased except when the pH of the intestinal contents fell below 2.5; then it was inhibited. There was an actual increase in the total amount of pepsin secreted and not merely an increase in concentration. This was well illustrated by the series of experiments in one dog (table 1) where an increase in total output was observed during acid instillation even though the volume of secretion remained the same or was even depressed.

In all experiments during the period following acid instillation, when there was an increased rate of secretion and the intestinal pH was above 2.5, the total output of pepsin was increased (tables 1 and 2). The increased pepsin output resulted in a pronounced increase in the concentration of pepsin in the gastric juice, particularly during the first hour or two following cessation of acid instillation in the intestine; subsequently, due to the great volume of juice secreted, the concentration fell off somewhat.

To be effective the volume of acid instilled had to be fairly large. Sixty cubic centimeters during 30 minutes was without effect (table 2) but 180 cc. during one hour produced a significant increase in pepsin secretion. Distilled water and 0.9 per cent NaCl had no pepsinogenic effect (table 2).

Seven test-meal experiments, three of which included intestinal infusion of acid, were performed on the Heidenhain-pouch dog. In two of the acid experiments the pepsin output was increased while in the third it remained unchanged. These results, obtained on but one animal, while suggestive, must be regarded as inconclusive.

Influence on pepsin secretion in response to insulin. A standard dose of 14 units of crystalline insulin (Mulford) was administered subcutaneously. During the secretion period which followed, acid was infused into the intestine as described above. The output of pepsin during the period of actual infusion usually was not greater but less than during the corresponding control period. However, in all these experiments the intestinal pH was lower than 2.0 and the volume of secretion was also decreased. Undoubtedly the threshold level for inhibition of secretion of pepsin, as observed in the test-meal experiments, was reached. When the intestinal pH was between 2.5 and 4.0, as it was during the period of increased secretion following the instillation of acid, both the concentration and the output of pepsin were increased.

Influence on pepsin secretion in response to histamine. Subcutaneous histamine injections repeated at ten-minute intervals at a standard dosage level of 2.5 mgm. histamine phosphate per hour resulted in a continuous secretion of gastric juice at a rate which was reproducible in each experiment.

Under histamine stimulation little pepsin is secreted from a gastric pouch. Under ordinary conditions, during the time that secretion occurs from the pouch, the intestinal pH is usually below 2.0 because of the entrance of undiluted gastric juice from the main stomach into the intestine. Since this low pH value is within the critical level observed for inhibition of pepsin secretion, it seemed possible that the low pepsin values might be due to inhibition from the intestine. To test this possibility, experiments were performed with the gastric cannula open so that the gastric juice was drained to the outside instead of into the

intestine. In control experiments with the stomach draining the pH of intestinal contents was usually above 4.0. When acid was instilled the intestinal pH ranged from 3.5 to 1.7. However, both during and following acid instillation the pepsin concentration, compared with control experiments, was neither increased nor decreased but remained uniformly as low as it did in the control experiments.

DISCUSSION. These experiments demonstrate that under certain conditions the instillation of acid into the small intestine provokes the secretion of pepsin by the chief cells of the stomach. The chief controlling factor appears to be the pH of the intestinal contents which is attained. Our results show pepsin secretion to be stimulated only when the intestinal pH is between about 6.5 and 2.5. On the other hand, when the pH is below about 2.5, the secretion of pepsin in response to food or insulin is inhibited as is the secretion of acid.

The pepsigogue effect of acid instillation into the small intestine was not manifest during gastric secretion provoked by histamine, in sharp contrast with the results obtained on feeding or administration of insulin. Food and insulin in themselves stimulate the chief cells while histamine is believed to stimulate selectively the parietal cells. The chief cells may require to be excited by some additional means in order that the pepsigogue stimulus from the intestine may become effective. On the other hand, Alley (1935) has reported that the secretion of pepsin which is evoked by sham feeding may be inhibited by histamine. The possibility remains that the secretion of pepsin provoked by acid in the intestine is likewise inhibited by histamine.

The number of experiments on the Heidenhain-pouch dog were insufficient to answer definitely the question whether the pepsigogue effect of introducing acid into the intestine involves a nervous reflex or a humoral agent such as the "pepsigogue" of Babkin and Komarov (1941).

Since it has been demonstrated that a stimulus applied in the intestine may increase the secretion of pepsin by the gastric glands, the way is open for consideration of a possible pepsigogue effect of other stimulants which participate in the intestinal phase of gastric secretion.

SUMMARY

Under certain conditions introduction of acid into the small intestine provokes the secretion of pepsin from the stomach. This pepsigogue effect of acid instillation is absent following the administration of histamine.

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THE LOW INCIDENCE OF ADRENAL ALTERATIONS IN BLACK RATS ON FILTRATE FACTOR DEFICIENT DIETS¹

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This investigation is concerned with the nature of the adrenal changes occurring in black or brown rats maintained on diets deficient in the filtrate factors of vitamin B. The study was prompted by the fact that although numerous reports of adrenal pathology in rats fed such diets have been published (1-6), the individual groups of animals have been limited in number, the results have shown considerable variation in the incidence and character of the adrenal lesions, and the deficient diets used have varied in certain respects, particularly as regards nicotinic acid and choline. In the present study a total of 284 black or brown rats were fed the filtrate factor deficient diet. The rats were divided into 5 groups according to the supplements of nicotinic acid or choline or both that were added to the diet. Seventy-four rats served as controls, receiving the filtrate factors in the form of a rice bran concentrate² alone or with supplements of nicotinic acid or choline as given to the deficient rats. In view of the fact that atrophy and necrosis of the adrenal cortex have been among the commonest of the adrenal lesions reputedly associated with filtrate factor deficiency, we have also determined the serum chloride levels in the various groups of rats studied.

PROCEDURE. Rats of the Long-Evans strain, bred in the laboratory, were used in the experiments. The filtrate factor deficient diet consisted of casein 22 per cent, sucrose 64 per cent, primex 9 per cent, and salt mixture 5 per cent; 1.9 cubic centimeters of cod liver oil was added to 100 grams of this mixture. In addition, each rat was given daily 15 mcgm. each of pyridoxine and thiamin chloride, and 45 mcgm. of riboflavin (table 1). For the control rats the same diet was used and to each 100 grams of diet 15 cc. of the rice bran concentrate was added as a source of the filtrate factor (table 2). Five groups of rats on the deficient diet (table 1) and 5 groups of rats on the control diet (table 2) were studied.

Group 1, consisting of 108 rats, received the filtrate factor deficient diet without nicotinic acid or choline. Fifty-four rats were kept on the low salt intake (0.064 per cent) and 54 received 1 per cent NaCl solution ad lib. Group 2, consisting of 60 rats, received the deficient diet and a daily supplement of 0.2 mgm. of nicotinic acid. Thirty-two rats of this group were on the low salt intake and 28 received the 1 per cent solution of NaCl. The 3 remaining groups on deficient diets received the 1 per cent solution of NaCl. In addition, group 3 (28 rats) received a daily supplement of 1 mgm. of nicotinic acid; group 4 (45 rats) received a daily supplement of 2 mgm. of choline and group 5 (43 rats)

¹ This investigation was aided by a grant from the Josiah Macy, Jr. Foundation.

² The rice bran concentrate was supplied by the Galen Co. of California.

received both choline and nicotinic acid (2 mgm. choline and 1 mgm. nicotinic acid). The duration of the experiments varied as indicated in the tables. The results in control rats, receiving the diet supplemented with rice bran extract (containing the filtrate factors) plus the supplements of nicotinic acid or choline, are reported in table 2.

The incidence of adrenal lesions is given for the rats autopsied in each group. When rats were not killed but died as a result of the deficiency, postmortem

TABLE 1

Summary of results on rats maintained on filtrate factor deficient diets with or without added nicotinic acid and choline

	GROUP 1		GROUP 2		GROUP 3	GROUP 4	GROUP 5
	A	B	A	B			
Number of rats.....	54	54	32	28	28	45	43
Number of rats autopsied*.....	52	46	29	20	26	44	25
Daily supplement							
Nicotinic acid (mgm.).....	None	None	0.2	0.2	1.0	None	1.0
Choline (mgm.).....	None	None	None	None	None	2.0	2.0
Salt intake.....	Low†	High‡	Low	High	High	High	High
Duration of experiment (days)....	67-141	77-138	53-131	50-131	45-93	56-91	45-96
% Died.....	17	24	19	43	60	35	53
% Incidence of graying.....	100	100	100	100	100	100	100
% Incidence of severe graying.....	61	32	65	57	64	41	49
% Dermatitis.....	7	6	16	32	11	4	12
% Alopecia.....	54	56	50	68	50	51	51
% Superficial hemorrhage.....	63	31	31	43	40	65	30
Avg. gain in weight (grams).....	55	31	59	33	29	19	15
% Gaining weight.....	100	96	100	100	93	84	77
Adrenal pathology†							
% Lipid depletion.....	36	35	17	20	50	23	36
% Atrophy.....	0	0	0	0	8	5	20
% Necrosis.....	0	9	3	15	15	9	16
% Hemorrhage.....	0	0	3	5	8	9	4
Serum chloride avg. (M Eq/l).....	98	99	98	99	98	99	99
Serum chloride range (M Eq/l)....	86-104	89-106	88-106	95-108	94-101	93-102	94-102
Number of rats determined.....	29	19	22	12	15	31	18

* When autopsies were not done it was because of autolysis.

† Based on number of rats autopsied.

‡ Low salt intake consisted of 0.06% NaCl ad lib.

§ High salt intake consisted of 1% NaCl ad lib.

autolysis in some animals did not permit satisfactory histological examination. The viscera, including the heart, lungs, liver, kidneys, spleen, pancreas and adrenals, were regularly examined. Tissues were fixed in Bouin's fluid for 24 hours, dehydrated and embedded in paraffin. Sections were stained with hematoxylin and eosin or with Goldner's modification of Masson's Trichrome Method. In addition, frozen sections were made from one half of an adrenal from each of 25 animals, and osmic acid or Sudan IV stains were applied and polariscopic examination performed.

Serum chloride determinations were made in 146 of the rats on the deficient diet and in 42 of the control rats just prior to sacrifice. The blood was withdrawn from the tail of the rat and the determinations were made in duplicate by the microtitration method.

RESULTS. The 108 rats in group 1 (filtrate factor deficient) (table 1) were sacrificed after periods varying from 67 to 138 days to observe what effect the duration of the deficiency might have on the adrenal glands. The only change observed in the rats in this group was moderate lipid depletion that occurred in 35 per cent of the rats. It was not influenced by the salt intake and the incidence was not greater in rats kept on the diet for longer periods. No significant degree of histologic atrophy of the adrenal cortex was found, but in 4 rats (9 per cent) on the high salt intake there was bilateral coagulative necrosis

TABLE 2
Results on control rats

	GROUP 1		GROUP 2		GROUP 3	GROUP 4	GROUP 5
	A	B	A	B			
Number of rats.....	15	14	9	9	7	12	8
Salt intake.....	Low*	High†	Low	High	High	High	High
Daily supplement							
Nicotinic acid (mgm.).....	None	None	0.2	0.2	1.0	None	1.0
Choline (mgm.).....	None	None	None	None	None	2.0	2.0
Duration experiment (days).....	69-115	77-110	91-115	108-111	92-93	85-91	78-96
% Died.....	0	0	0	0	0	0	0
% Incidence of graying.....	0	0	0	0	0	0	0
Weight changes (% gained).....	100	100	100	100	100	100	100
Average grams gained.....	122.6	128.4	124.3	124.1	124.0	164.0	200.3
Adrenal pathology.....	None	None	None	None	None	1 rat	None
Serum chloride avg. (M Eq/l)....	99	100			99	97	99
Serum chloride range (M Eq/l)....	96-103	93-109			98-100	92-100	96-102
Number of rats determined.....	9	12			4	11	6

* Low salt intake consisted of 0.06% NaCl ad lib.

† High salt intake consisted of 1% NaCl ad lib.

of the adrenals. None was found in the rats on the low salt intake, so that for the groups as a whole, the incidence was only 4 per cent. Moderate lipid depletion occurred in conjunction with necrosis in 1 rat. Of the rats in which adrenal cortical necrosis was present, 1 died on the 66th day of the experiment and the other 3 were sacrificed after 81, 106 and 119 days of the deficient diet. Hemorrhage of the adrenal cortex did not occur in any of the rats.

The question arose as to whether necrosis and hemorrhage might have been present in the rats found dead and in which histological examination was impossible due to autolysis of the adrenals. Assuming that all such animals also had necrosis, the incidence for the rats on the low salt intake would have risen to 3.5 per cent (2 rats), and for the rats on the high salt intake it would have been 22 per cent (12 rats), or a total of 14 of the 108 rats observed.

Graying of the fur occurred in all animals whether the salt intake was high or low. It was most severe in rats on the low salt intake. The average onset of graying was after 23 days of the diet, with a range from 17 to 40 days. Other changes observed were alopecia, dermatitis and superficial hemorrhages in the skin. The latter were more common in rats on the low salt intake. All rats gained weight during the course of the experiment; the gain varied from 2 to 120 grams. The control rats (table 2) naturally gained much more weight (average 122.6 grams) and exhibited no graying. No changes were found in the adrenals of any of the control animals.

In group 2 (60 rats) the deficient diet was supplemented with 0.2 mgm. of nicotinic acid daily. In 32 rats the salt intake was low, and the remainder received 1 per cent NaCl solution ad lib. The duration of the experiments was from 50 to 131 days. Nineteen per cent of the rats on the low salt intake and 43 per cent of the rats on the high salt intake died during the course of the experiment, but more than half of the rats that died had survived on the diet for 100 days or longer. In this group some lipid depletion of the adrenal cortex was present: 17 per cent in the rats on the low salt intake and 20 per cent on the high salt intake. The incidence for the entire group was very slightly lower than in group 1. Bilateral necrosis and hemorrhage of the adrenal cortex was observed in 1 rat on low salt intake and this was an animal that died on the 128th day of the diet. Necrosis of the adrenal cortex was present in 3 rats on high salt intake; 1 was a rat that died after 70 days of the diet. The other 2 rats were sacrificed after 125 and 131 days. Slight hemorrhage was also present in the adrenal cortex of one rat that had necrosis. Histologic atrophy was not observed in any adrenal glands. Again, had necrosis and hemorrhage been present in the adrenal glands that were not examined histologically, the incidence of this change would have been increased to 12.5 per cent (4 rats) in the rats on the low salt intake, and 39 per cent in the rats on the high salt intake. Graying of the fur occurred in all the rats and was more severe than in group 1. The other changes again included alopecia, dermatitis and superficial hemorrhages. The incidence of dermatitis was twice as great in the rats on high salt intake (table 1).

The 28 rats in group 3 received the deficient diet plus a larger daily dose of nicotinic acid (1 mgm.). One per cent NaCl was given ad lib to these rats. The duration of the experiment varied from 45 to 93 days. The shorter duration, as compared to groups 1 and 2, was due to the fact that 60 per cent of the animals died after shorter periods on this modification of the diet. Lipid depletion of the adrenal cortex was present in 50 per cent. Its occurrence did not bear any relation to the duration of the experiment. Hemorrhage, together with extensive necrosis of the adrenal cortex, occurred in 2 animals. In 2 other animals there was less extensive necrosis of the cortex. Two additional rats in this group were not studied histologically; had hemorrhage and necrosis been present in these rats, the incidence would have been increased to 21.5 per cent (6 rats). Atrophy of the cortex occurred alone in 1 rat and accompanied lipid depletion in 1 other rat. Most rats gained weight during the experimental

period. All animals showed graying of the fur and it was severe in 64 per cent. Dermatitis was infrequent, but the incidence of alopecia and superficial hemorrhages was about the same as for other groups.

The 44 rats in group 4 received the deficient diet plus 2 mgm. of choline daily. Thirty-five per cent of the rats died after 56 to 85 days. The rest of the animals were sacrificed after 80 to 91 days on the diet. Lipid depletion of the adrenals was present in 10 (23 per cent) of the animals and did not occur in conjunction with any other pathological changes. This was also true for the atrophy, which was found in only 2 rats (5 per cent). Necrosis, together with hemorrhage of the adrenal cortex, was present in 4 (9 per cent) of the animals. Had this lesion been present in the 1 rat that had autolysis when found dead, the incidence would have been increased to 12 per cent (5 rats).

Again graying occurred in all animals and was severe in 41 per cent (18 rats). Less dermatitis was observed. Alopecia was present as in the preceding groups, but there was an increase of the number of animals showing superficial hemorrhages of the skin (table 1), as compared to groups 2 and 3. Eighty-four per cent of the animals gained weight during the first 6 weeks of the experiment, but lost weight during the latter part of the experiment.

The rats in group 5 (43 rats) received the deficient diet plus 1 mgm. of nicotinic acid and 2 mgm. of choline daily. The animals were sacrificed or died after 45 to 90 days on the diet. The earlier time of sacrifice was necessary, as in groups 3 and 4, because the animals became debilitated sooner than in groups 1 and 2. They all lost weight during the end of the experimental period, and the total gain of weight for the experimental period was slight. Severe graying occurred in all. Thirty-six per cent (9 rats) showed lipid depletion of the adrenal cortex, and atrophy was observed in 5 rats (20 per cent), necrosis in 4 rats (16 per cent), and hemorrhage in 1 rat among the latter. Atrophy accompanied necrosis in 1 adrenal and was an independent finding in the others. Had necrosis occurred in the 18 rats in which pathological examination was impossible, the incidence of this lesion would have been increased from 16 per cent (4 rats) to 51 per cent (22 rats).

The serum chloride values are not reported in detail but the average values and the ranges are given in tables 1 and 2. The chloride level of the serum determined in 42 of the control rats varied from 92 to 109 M Eq/1. In only 5 rats (11 per cent) were the values below 96 M Eq/1. The latter value we considered the lower level of normal for rats of this age. Selye and Dosne (7) reported plasma values of 557 mgm. per cent NaCl for normal young albino rats, which is about 96 M Eq/1.

In the deficient rats in group 1-A (filtrate factor deficient plus low salt intake) 29 chloride determinations were made. The serum chloride levels were below 96 M Eq/1 in 3 rats and in each case the adrenal cortex in these animals showed moderate lipid depletion. In the rats in group 1-B that received the deficient diet plus 1 per cent NaCl, the serum chloride level was low in 3 of the 19 rats in which it was determined. In only one instance was this associated with lipid depletion of the adrenal cortex. In the rats in group 2-A (filtrate factor deficient,

low salt plus 0.2 mgm. nicotinic acid) 22 chloride determinations were made. The level was decreased in 3 animals and in 1 of these animals there was moderate lipid depletion of the adrenal cortex. In the rats in group 2-B, on the same diet except for the high salt intake, 12 chloride determinations were made, and in 2 rats the serum level was lowered. In 1 of these there was moderate lipid depletion of the adrenal cortex. In the rats in group 3 receiving the deficient diet plus 1 mgm. of nicotinic acid daily and 1 per cent NaCl ad lib, 15 chloride determinations were made and in 4 instances the level was lowered. In 2 of these animals there was necrosis of the adrenal cortex and in the other 2 there was moderate lipid depletion. In group 4, 31 chloride determinations were made and the values were low in 4 rats. This was accompanied by lipid depletion in one and by moderate atrophy of the adrenal cortex in another instance. In group 5 (filtrate factor deficient plus 1 mgm. nicotinic acid and 2 mgm. choline) 18 chloride determinations were made and the level was lower than normal in 1 rat; this was not accompanied by any histologic change in the adrenal cortex.

Of the 146 deficient rats in which serum chloride levels were determined, the values were below normal in 20, and in 12 of these they were associated with either lipid depletion of the adrenal cortex or necrosis. However, in 44 of the animals in which lipid depletion of the cortex was observed, the serum chloride levels were normal. Lipid depletion alone apparently need not be associated with changes in the chloride level.

DISCUSSION. The most frequent pathological change observed in the adrenal gland was lipid depletion of the cortex. In fact, in the rats on the deficient diet alone with the low salt intake (group 1-A), this was the only change observed. In other groups lipid depletion was never present in more than 50 per cent of the adrenals and the incidence varied from 17 to 50 per cent in the various groups. The estimate of lipid depletion was based on the absence of lipid vacuoles in the cortical cells. Masked droplets, too fine to be seen in ordinary sections, were not encountered in preparations examined after applying osmic acid or Sudan IV to frozen sections. Anisotropic lipid was not regularly sought but when present was always found in adrenals with vacuolated cortical cells.

Necrosis with or without hemorrhage was found in the adrenals of a few animals in all the groups, with the exception of group 1-A. In none of the groups, however, was the incidence greater than 16 per cent and it occurred principally in rats receiving nicotinic acid. Atrophy of the cortex was an infrequent finding except in the rats receiving both nicotinic acid and choline, in which 20 per cent of the adrenals showed some atrophy. Calculating the incidence of adrenal necrosis and hemorrhage for the entire group of 242 rats in which the adrenals were examined, the incidence of this pathologic change was only 8.2 per cent.

We are unable to explain the sharp discrepancy in adrenal lesions between our results and those reported by others. The adrenal changes we observed were slight. Serious lesions were so rare as to suggest that they were due to some mechanism other than filtrate factor deficiency. One possible exception may have occurred in group 4 in which the deficient diet was supplemented by

both choline and nicotinic acid. The animals in this group were short lived and many were found dead. If adrenal necrosis had been present in all the animals that died, then the incidence of the lesion in this group would have been greater than that found in any of the other groups. In the absence of histologic proof, however, this continues to be a matter of speculation. If accepted as theoretically possible, the incidence would still be below that reported by most other investigators.

Graying of the fur, which is the characteristic change reported in rats on filtrate factor deficient diets, was present in all of the deficient animals in this study. In addition, alopecia, cutaneous hemorrhages and, to a less extent, dermatitis occurred in all groups but not in all the animals.

SUMMARY

Five groups of rats, totaling 284 animals, were fed diets deficient in the filtrate factors of vitamin B. The groups were divided so that one received the deficient diet with no supplements of nicotinic acid or choline, and the others received either 0.2 or 1 mgm. of nicotinic acid daily, or 2 mgm. of choline, or both nicotinic acid and choline. In the first 2 groups half of the rats were given a low salt intake. All the other groups received 1 per cent NaCl ad lib.

All animals developed graying of the fur. This was most severe in rats on low salt intake and in rats receiving nicotinic acid daily. Dermatitis, alopecia and superficial hemorrhages of the skin occurred to a varying degree in all groups.

The most common change observed in the adrenal cortex was lipid depletion, but this did not occur in more than 50 per cent of the animals in any group. In rats maintained on the filtrate factor deficient diet and not supplemented with nicotinic acid or choline, lipid depletion was the only significant pathological change found in the adrenals. The addition of nicotinic acid to the diet increased the incidence of necrosis of the adrenal cortex slightly. When both nicotinic acid and choline were added, there was an increase of the incidence of atrophy. When only choline was added to the diet, the adrenal change consisted of lipid depletion with a very small incidence of atrophy, necrosis and hemorrhage.

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POSITIVE INJURY POTENTIALS OF THE STOMACH¹

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The fact that an injured region of muscle or nerve is electro-negative in the external circuit to an uninjured region (a negative injury potential) has been demonstrated many times. Osterhout and his associates (1) have shown that under certain conditions injury produces a positive injury potential in some of the coenocytic algae. The concept of the negative injury potential, however, has become so well established that it is assumed that the coenocytic algae are unusual and that in general injury produces a negative injury potential in both plant and animal tissues (2, 3). Abramson (4) in his theory that the injury potential influences the movements of leucocytes into an injured region assumes the injury potential to be negative. Recently, however, Burr and his colleagues (5, 6) and Burrows, Iball and Roe (7) found that injury produces a positive injury potential in the skin of certain mammals. On the basis of these reports it would seem that injury to animal tissues is not always accompanied by a negative injury potential and that further studies on other tissues would be of interest.

According to the classical concept of the origin of the negative injury potential in muscle and nerve, injury causes a marked decrease in the magnitude of the potential at the site of injury, and the potential at the uninjured region which is directed outward is responsible for the uninjured region being positive in the external circuit to the injured region. Osterhout found under the conditions in which positive injury potentials were observed in the coenocytic algae that the potential was directed inward toward the sap (opposite to that of nerve and muscle) and that injury abolished this potential. According to this concept injury decreases the potential at the point of injury and the orientation of the injury potential is dependent on the orientation of the inherent potential of the tissue, being negative when this potential is directed outward and positive when directed inward. Assuming that this principle is applicable to such relatively complex tissues as the stomach one would expect a positive injury potential with electrodes placed on the mucosa since the potential across the stomach is directed from the mucosa to the serosa (8) (the serosa being positive in the external circuit to the mucosa). One of the purposes of the present paper is to demonstrate that such is the case. Studies in the past on the origin of injury potentials have been confined to a demonstration of a qualitative relation between the decrease in potential at the site of injury and the production of the injury potential. An attempt has been made in the present work to investigate the quantitative relationship between these potentials.

METHODS. The stomach was exposed through an abdominal incision in per-

¹ This investigation was aided by a grant from the Joseph E. Seagrams and Sons Company.

nostonized dogs² (80 mgm. per kgm. subcutaneously). A longitudinal cut was made in the stomach midway between the lesser and greater curvatures. The flap of stomach was placed on a lucite platform with the serosal side downward. The lucite platform contained two Zn-Zn acetate agar, Ringer agar electrodes. The area of the Ringer agar surface of these electrodes in contact with the serosa was 3 cm². Two other similar electrodes were placed in contact with the mucosa directly opposite the serosal electrodes. The Ringer agar of these electrodes extended several millimeters beyond the glass tubes and the pressure exerted by these electrodes on the stomach was less than 8 cm. of water. This arrangement is shown diagrammatically in figure 1. The opening in the lucite platform between the serosal electrodes (1.5 cm.) prevented short circuiting of these electrodes through fluid which might otherwise accumulate under the serosa.

Potential differences were measured between electrodes M₁ and S₁, M₁ and M₂, M₂ and S₂, and S₁ and S₂ before and after injury. Boiling Ringer's, ether and ethyl alcohol were used to produce injury. In referring to the potential difference between two electrodes a positive sign means that the electrode represented by the first letter is positive in the external circuit to the electrode represented by the second letter.

The potential differences were measured with either a Hindle string galvanometer or with a potentiometer. When the potentials were measured with the string galvanometer the leads from the electrodes were connected in series with the galvanometer and a resistance of approximately 60,000 ohms. It was found that the change in resistance of the electrodes plus stomach that occurred after injury was smaller than that necessary to produce an observable difference in the deflection of the string.

A total of ten dogs was used in these experiments.

RESULTS. In figure 1A, a typical experiment, it can be seen that injury to the region of electrode M₁ was followed by a marked increase in positivity of this region with respect to an uninjured region (M₂). Injury in this experiment was produced by directing a fine stream of very hot Ringer's at the region of electrode M₁.

In control experiments it was found that washing the region under one electrode with Ringer's solution at body temperature produced very little change in the potential difference, usually less than 2 mv. and that applying heat, alcohol or ether to the dead stomach produced changes less than 1 mv.

Positive injury potentials associated with a decrease in the magnitude of the potential across the stomach at the point of injury. Figure 1B shows the results of a typical experiment in which the potentials were measured (with the potentiometer) between the four regions mentioned above. It can be seen that the mucosa is negative in the external circuit to the serosa at both regions (M₁S₁ and M₂S₂). This was found to be true before injury in all of the experiments. At the time indicated by the first arrow 2 cc. of 95 per cent ethyl alcohol were applied to the region of electrode M₁. This was followed by a marked decrease in the magni-

² The Riedel-deHaen Company, New York, kindly supplied the pernoston used in these experiments.

tude of the potential across the stomach at this region (M_1S_1) and a marked increase in the positivity of M_1M_2 (positive injury potential.) At the time indicated by the second arrow alcohol was applied to the region of M_2 . This was followed by a marked decrease in the magnitude of M_2S_2 and an increase in positivity of M_2 with respect to M_1 (i.e., M_1M_2 decreased in magnitude). It should be noted that there was very little change in S_1S_2 after injury. Care was taken to make sure that the electrodes S_1 and S_2 were not shunted by a layer of fluid outside the stomach.

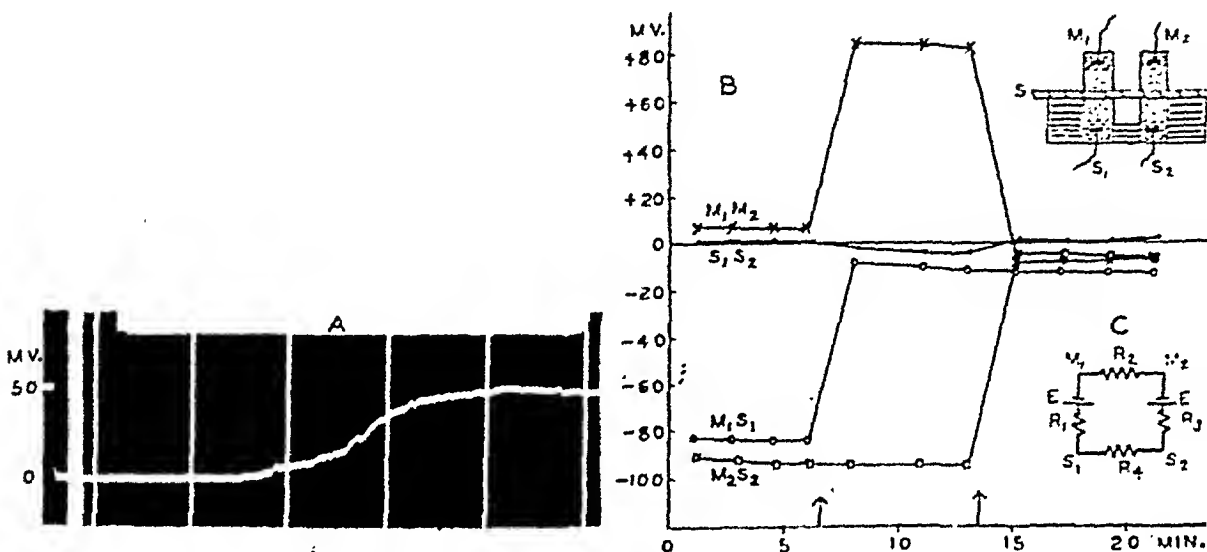


Fig. 1A. String galvanometer record of M_1M_2 . Boiling Ringer's solution applied at first time line after beginning of white line at top of record. Time interval between two vertical lines is 4 sec. Upward deflection means M_1 has become more positive in external circuit to M_2 . At beginning of record galvanometer calibrated with 50 mv.

Fig. 1B. M_1 and M_2 represent two electrodes on the mucosal surface and S_1 and S_2 two electrodes on serosal surface. S represents the stomach. Stippled areas represent 2 per cent Ringer agar; small horizontally lined areas, saturated Zn acetate agar. The longer horizontal lines represent the lucite platform.

Fig. 1C. Circuit diagram. M_1 , M_2 , S_1 and S_2 represent regions where the corresponding electrodes are placed. R_1 and R_2 , resistances across stomach from mucosal to serosal surfaces. R_3 and R_4 represent longitudinal resistances of the stomach. E represents potential across stomach.

Relation between the magnitude of the injury potential and the magnitude of the changes in potential across the stomach. In table 1 the results of the experiments on the effect of injury on the mucosa in which the four potentials were measured are summarized. The values of the potentials 2 to 6 min. after injury were subtracted from their values immediately before injury. The magnitudes of the potentials across the stomach were in some cases as low as 40 mv. immediately after placing the electrodes on the stomach. In the absence of further experimental procedure the potentials increased and in about one hour reached a relatively constant level in the neighborhood of 80 mv. The effect of injury on the potentials was determined at various potential levels.

Examination of columns 4 and 5 reveals that injury always increased the positivity of the mucosa with respect to the serosa at the site of injury (a decrease in the magnitude of the potential across the stomach). Presumably because of the tendency of the potentials to increase with time there was usually a small change in the magnitude of the potential across the stomach at the uninjured region

TABLE 1

EXPT.	S.I.	I.A.	$\Delta M_1 S_1$	$\Delta M_2 S_2$	$\Delta S_1 S_2$	$\Delta M_1 M_2$	$\Delta M_1 S_1 - \Delta M_2 S_2$	D_1	$\Delta M_1 M_2$ CALC.	D_2
1	M ₁	B.R.	+35	+0.5	-3	+33	+34.5	+1.5	+31.5	-1.5
2	M ₁	A	+67	-2	-5.5	+63.5	+69	+5.5	+63.5	0
3	M ₂	A	0	+69.5	-1.5	-71.5	-69.5	-2	-71.0	-0.5
4	M ₁	A	+42	+0.5	0	+43.5	+41.5	-2	+41.5	-2
5	M ₂	A	-1.5	+59	+6	-56	-60.5	+4.5	-54.5	-1.5
6	M ₁	A	+24	-2	-2	+23	+26	+3	+24	+1
7	M ₂	A	-2	+52	+1.5	-55	-54	-1	-52.5	-2.5
8	M ₁	A	+76.5	-3.5	-3	+76.5	+80	+3.5	+77	+0.5
9	M ₂	A	-1.5	+91.5	+3	-90	-93	+3	-90	0
10	M ₁	E	+31	-3	-0.5	+32	+34	+2	+33.5	+1.5
11	M ₂	E	+1	+10.5	+2.5	-9	-9.5	+0.5	-7	-2
12	M ₁	E	+7.5	-3	-0.5	+9	+10.5	+1.5	+10	+1
13	M ₁	E	+11.5	-1	0	+11	+12.5	+1.5	+12.5	+1.5
14	M ₁	A	+41.5	+0.5	0	+40.5	+41	+0.5	+41	+0.5
15	M ₁	E	+25	-4	0	+27.5	+29	+1.5	+29	+1.5
16	M ₂	E	-1	+36	+1	-35	-37	+2.0	-36	+1
17	M ₁	$\frac{1}{2}$ A	+27	-5.5	0	+30.5	+32.5	+2.0	+32.5	+2.0
18	M ₁	A	+59.5	-2	-2.5	+60.5	+61.5	+1	+59	-1.5
19	M ₂	$\frac{1}{2}$ A	-1	+48.5	-0.5	-50.5	-49.5	-1	-50	-0.5
20	M ₂	A	-3	+40	-2.5	-44.5	-43	-1.5	-45.5	+1
Average								+1.30		-0.02
S.D.								± 2.03		± 1.36

Column S.I. gives the site of injury; column I.A. gives the agent used to produce injury; B.R. refers to boiling Ringer's; A to 95 per cent ethyl alcohol; $\frac{1}{2}$ A to equal parts of 95 per cent ethyl alcohol and Ringer's; E to ether. Columns 4, 5, 6 and 7 ($\Delta M_1 S_1$, $\Delta M_2 S_2$, $\Delta S_1 S_2$, and $\Delta M_1 M_2$) represent the changes in potential after injury; a positive sign means that the potential of the electrode represented by the first letter has become more positive in the external circuit with respect to the electrode represented by the second letter and vice versa. Column 8 gives the sum of the changes in the potentials across the stomach. Column 9 represents the difference in absolute magnitudes of the values in columns 7 and 8. Column 10 gives the value of $\Delta M_1 M_2$ calculated from the changes in the other potentials. In column 11 the differences in absolute magnitudes between columns 10 and 7 are given. The standard deviations are given below the averages in columns 9 and 11.

(column 4 when the region of M₂ was injured and column 5 when the region of M₁ was injured). The average increase after injury in the potential across the stomach at the uninjured region was 1.67 mv. (S.D. = ± 1.65 mv.), i.e., average of the values across the uninjured portion of the stomach given in columns 4 and 5 = -1.67 mv. Column 8 gives the sum of the changes in the potentials at the injured and uninjured regions. It can be seen from the table that the values

in column 8 are approximately equal to the magnitudes of the injury potentials (column 7). Since the changes in the magnitudes of the potentials at the uninjured region were relatively small it is also true that the injury potentials are approximately equal to the changes in the potentials at the site of injury (column 4 or 5). It should be pointed out that the negative values of $\Delta M_1 M_2$ are correlated with injury to M_2 and indicate that M_2 , the site of injury, has become more positive with respect to M_1 (a positive injury potential). Column 6 shows that the values of $\Delta S_1 S_2$ are relatively small compared to those of $\Delta M_1 M_2$.

Because of the presence of rapid fluctuations of small magnitudes (see fig. 1A) it was not possible to measure the potentials with the potentiometer with a greater accuracy than about 0.5 mv. The order of magnitude of the error involved in comparing the potentials due to these variations can be determined by calculating $\Delta M_1 M_2$ from the other potentials and comparing this value with the measured value of $\Delta M_1 M_2$. From the classical laws of electrical networks it follows that

$$\Delta M_1 M_2 = \Delta M_1 S_1 - \Delta M_2 S_2 + \Delta S_1 S_2 \quad (1)$$

Column 10 shows the value of $\Delta M_1 M_2$ calculated in this way and column 11 shows the differences between these values and those of the measured values of $\Delta M_1 M_2$. In calculating the differences in column 11 no attention was paid to the signs in columns 7 and 10 since the sign depends upon whether the injured region is designated by M_1 or M_2 . The average value of the calculated values of $\Delta M_1 M_2$ was 0.02 mv. (S.D. = ± 1.36 mv.) less than the average of the measured values of $\Delta M_1 M_2$.

DISCUSSION AND CONCLUSIONS. The evidence presented above demonstrates that injury to the mucosa of the stomach produces a positive injury potential. The above findings are in accord with the essentials of the classical concept of the origin of the injury potential in nerve and muscle except that in the case of the stomach the potential is directed inward from the mucosa and injury therefore produces a positive injury potential. This work together with that of Osterhout (1) suggests that in any tissue with the potential directed inward from the surface injury would be expected to produce a positive injury potential. It is interesting to note that Burrows et al. (7) find that the potential in the skin of rats is directed inward from the surface. The data also demonstrate that the magnitude of the injury potential is approximately equal to the sum of the changes in potentials across the stomach or to the change in the magnitude of the potential across the stomach at the injured region.

The experimental results presented in the present paper can be readily interpreted on the basis of the equivalent circuit represented in figure 1C. The magnitude of the changes in $M_1 S_1$ and $M_2 S_2$ would determine the magnitude of the changes in $M_1 M_2$ and $S_1 S_2$. The actual values of $\Delta M_1 M_2$ and $\Delta S_1 S_2$ would depend on the relative values of resistances R_2 and R_4 . Since $\Delta M_1 M_2$ is relatively large compared to $\Delta S_1 S_2$ it is reasonable to conclude that R_2 is relatively large compared to R_4 .

The findings in the present paper together with those of Burr et al. (5, 6) and Burrows et al. (7) are not in accord with Abramson's (4) theory of the influence of the injury potential on the movements of leucocytes since according to his formulation a positive injury potential would cause the leucocytes to move out of the injured region. However, since a flow of current into an injured region must be accompanied by a flow of current out of the region, an injury potential irrespective of its direction if it influenced the movements of leucocytes, would tend to cause them to move into the injured region at one locus and out at another. Since Abramson has demonstrated *in vitro* that voltage drops which are probably no greater than voltage drops in living tissues appreciably influence the movements of leucocytes, the finding of positive injury potentials in the skin and stomach would not be in conflict with this essential point in the theory. It is obvious that a much more thorough knowledge of the patterns of current flow in tissues is desirable.

SUMMARY

Injury to a region of the mucosa of the dog's stomach produced a decrease in the potential across the stomach at the site of injury and an increase in the positivity of the injured region to an uninjured region of the mucosa (a positive injury potential). It was found that the magnitude of the injury potential was approximately equal to the magnitude of the changes in the potentials across the stomach.

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DYNAMICS OF COLLATERAL CIRCULATIONS^{1, 2}

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In studying peripheral resistance changes and vasomotor reactions we attempted to determine the blood flow through specific vascular beds by measuring either the flow into the artery supplying the region or the flow out of the vein draining it. The initial studies indicated, however, that either the inflow or the outflow may differ very considerably from the flow through the vascular bed whenever the pressure in the artery or vein differs from that in the corresponding collateral blood vessels. This discrepancy is due to the variable exchange of blood across the arterial and venous anastomotic channels between the vascular bed being studied and the collateral vascular beds. This paper presents an analysis of the magnitude of this exchange of blood under various conditions of perfusion in different vascular beds. It also discusses methods of interpreting the efficiency with which such anastomotic communications protect against sudden occlusion of the main arterial branch.

Anatomical considerations. The anatomical arrangement of the peripheral circulation may be illustrated schematically as shown in figure 1. The lower half of the figure represents the vessels in which vasomotor reactions are being studied. This we have called the cognate system.³ The upper half of the figure represents all adjacent vessels having arterial and/or venous anastomotic communications with the cognate capillary bed. These we have called the collateral systems.

Three cognate systems were studied:

1. The first of these was the capillary bed of the left quadriceps muscle supplied by the deep penetrating branches of the femoral artery. This vascular bed was studied by isolating a segment of the left femoral artery by two clamps, C2 and C3, placed respectively at the level of the inguinal ligament and just proximal to the saphenous branch, and perfusing by cannula IEA inserted into the left inferior epigastric artery. This segment of the femoral artery will hereafter be termed the cognate artery of the quadriceps muscle. The connections are illustrated in figure 2.

2. The second vascular bed studied was that of the skin over the medial aspect of the lower thigh and leg and the dorso-medial aspect of the paw. The cognate artery for this vascular bed was the saphenous branch of the femoral artery. This area was studied by perfusing the saphenous branch of the left femoral

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² A preliminary report of this paper was published in the Fed. Proc. 1: 32, 1942.

³ The word *cognate* (meaning born together) was borrowed from genealogy and is used in a similar sense to refer to a direct lineal path as contrasted to a collateral path.

artery, SA, by a cannula inserted into the femoral artery at LFA, as shown in figure 2, while the femoral artery was compressed centrally to the branch by clamp C3.

3. The third vascular bed was that of the skin and muscle of the lower part of the left hind leg. Its cognate artery was the popliteal artery. This vascular bed was perfused by way of a cannula inserted centrally into the saphenous branch, while the femoral artery was compressed by a clamp applied to the femoral artery just proximal to the saphenous branch.

Preliminary studies were made on these three cognate systems to determine the extent of the collateral systems which had anastomotic communications with them. This was done by cannulating as above for perfusion and by collecting the backflow from the cannula against atmospheric pressure. The arteries sup-

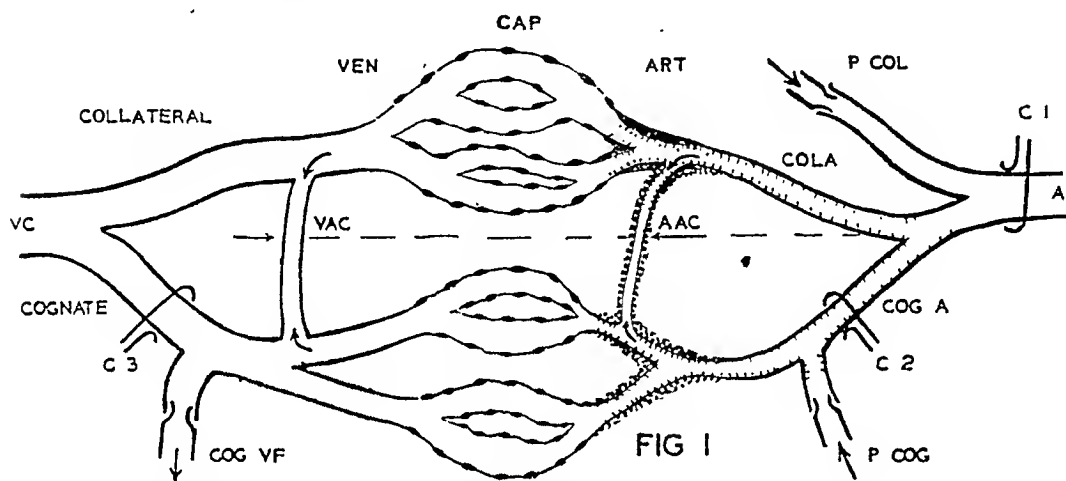


Fig. 1. Schematic diagram of the cognate and collateral systems. ART—arteries; CAP—capillaries; VEN—veins; P COL—cannula for perfusion of collateral arteries; A—aorta; COL A—collateral supply artery; COG A—cognate supply artery; AAC—arterial anastomotic channel; P COG—cannula for perfusion of cognate artery; VAC—venous anastomotic channel; COG VF, cannula for collecting and measuring venous outflow from cognate vein, the horizontal dot-dash line indicates division between cognate and collateral systems. C1, C2 and C3—clamps for compressing the vessels.

plying adjacent vascular beds were then progressively compressed until the backflow ceased. It was found that in order to eliminate satisfactorily the backflow from any of these three cognate systems it was necessary to compress all arteries leaving the aorta below the level of the renal arteries. This was most easily accomplished by compressing the aorta itself. In order, therefore, to study the effects of various pressures in the collateral arteries upon the flow into the cognate artery we routinely, in all subsequent experiments, compressed the aorta at this level and perfused the distal portion by way of a cannula inserted into the central stump of the right femoral artery, as indicated in figures 1 and 2.

I. EFFECTS OF COLLATERAL CIRCULATION UPON ARTERIAL INFLOW MEASUREMENTS. *Methods.* Flow into the cognate arteries of one or two of the above cognate systems was recorded in each of 20 dogs anesthetized with morphine and

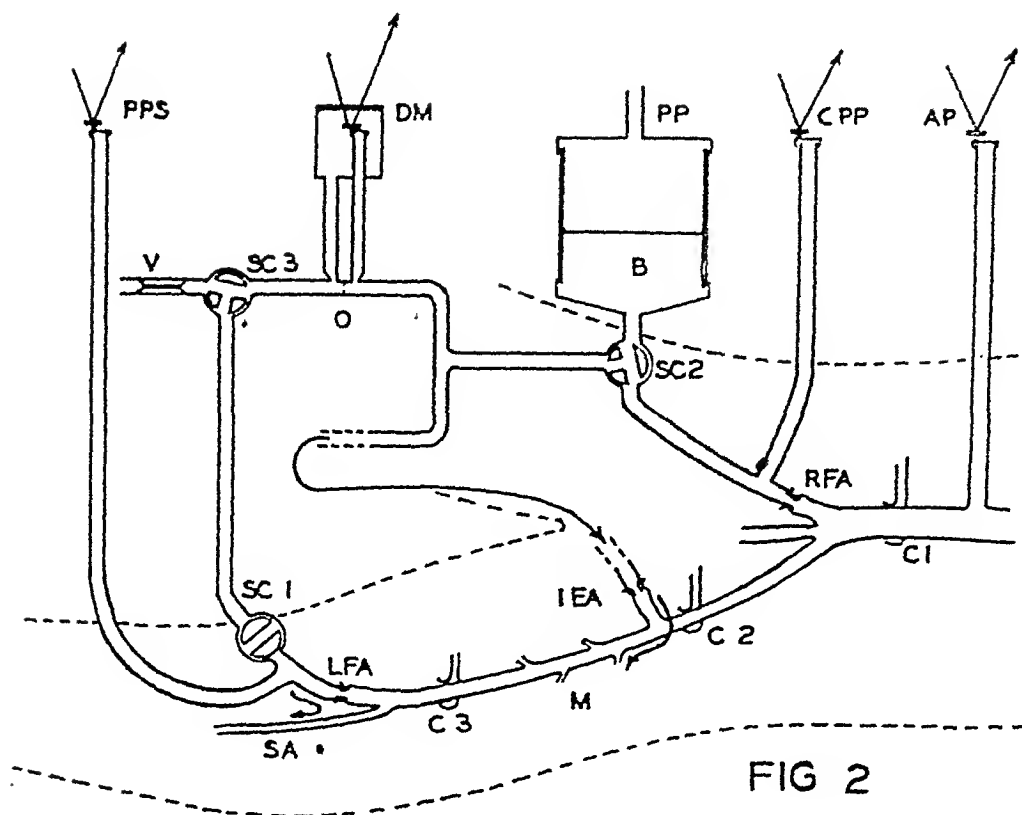


FIG 2

Fig. 2. Schematic diagram of apparatus and connections used for simultaneous perfusion of the cognate and collateral arteries. Blood entered reservoir *B* through the right femoral artery (*RFA*) and stopcock *SC2*. When 20 to 60 ml. of blood had been obtained the stopcock was closed and the air pressure above the blood (*B*) raised and maintained at the desired level during subsequent perfusion by a sensitive pressure-regulating system connected at *PP*. Blood for perfusion of the cutaneous artery (*SA*) flowed through orifice *O* and stopcocks *SC3* and *SC1* and thence into the cannula inserted into the distal end of the left femoral artery (*LFA*) while clamp *C3* was compressed. The perfusion pressure was recorded optically by manometer *PPS*, and the rate of flow through orifice *O* by differential manometer *DM*. Flow into the branches of the femoral artery (*M*) which supply the quadriceps muscle was recorded simultaneously by a similar system connected as indicated by the arrow to a cannula inserted into the inferior epigastric artery (*IEA*), while the femoral artery was compressed by clamps *C2* and *C3*. The collateral arteries were perfused through the connection with the right femoral artery (*RFA*) mentioned above while the aorta was compressed by clamp *C1*. The pressure at which the collateral arteries were perfused was recorded by manometer *CPP*. Central aortic pressure was recorded by manometer *AP* inserted by way of a carotid artery. Clamp *C1* was placed in most experiments by means of a retroperitoneal approach after dissecting the inguinal ligament from the symphysis pubis. In a few experiments a direct transperitoneal approach was used. The orifices were calibrated at the beginning and end of each set of determinations by driving blood from the reservoir through resistance *V* and collecting and measuring the rate of flow while recording the deflection of the recording light beam of the differential manometer. By recording the pressure drop across the resistance with manometer *PPS* the viscosity of the blood was simultaneously recorded. The details of the flow-meter, manometers and optical system are described elsewhere (1, 2, 3).

sodium barbital. The cognate and collateral arteries were always left in direct communication with the aorta except when they were being perfused. In each

experiment flow into the cognate artery was measured while it was being perfused with blood from a reservoir at each of a series of pressures from zero to above mean aortic pressure. Two flow measurements were made at each perfusion pressure: 1, while the collateral arteries were receiving blood directly from the aorta, and 2, while the collateral arteries were perfused from the reservoir at the same pressure as the cognate artery. Flow was recorded at each pressure for 5 to 20 seconds and the entire series of measurements was completed in approximately 20 minutes. The details of the perfusion apparatus in a typical experiment are described in the legend to figure 2. In all experiments after completion of dissections and before starting perfusion, the animals were given approximately 0.3 ml./kgm. of heparin solution⁴ initially, plus 0.1 ml./kgm./half-hour thereafter to prevent clotting.

Results. A. Blood flow into the cognate arteries supplying the quadriceps muscle.

1. *Flow into the cognate arteries when the collateral arteries were supplied from the aorta.* The results in all 10 experiments of this group were similar. Those from a typical experiment are plotted as line CPA in figure 3. This plot shows that at the lowest perfusion pressure, 10 mm. Hg, blood flowed back up into the reservoir at a rate of 16 ml./min. (maximum backflow, MBF). As the pressure in the reservoir was raised the backflow progressively decreased until at a pressure of 68 mm. Hg no flow occurred. This pressure we have designated the zero flow pressure, ZFP. At pressures above 68 mm. Hg inflow occurred, increasing progressively as the perfusion pressure was raised.

Since the lowest perfusion pressure was greater than the pressure in the veins it is obvious that the backflow could not have come from the veins; it must, therefore, have come from the collateral vascular beds. The progressive decrease in the rate of backflow from the cognate artery as the perfusion pressure was raised from 10 to 68 mm. Hg was evidently due, *a*, to a decreasing rate of flow from the collateral to the cognate arteries, and *b*, to an increasing rate of flow through the cognate bed. On this basis, the zero flow pressure would be that pressure at which the rate of flow from the collateral arteries across the anastomotic channels just equalled the rate at which the blood was flowing through the cognate bed.

2. *Flow into the cognate arteries when the collateral and cognate arteries were perfused at the same pressure.* The results of this method of perfusion of the vascular bed of the quadriceps muscle were essentially similar in all experiments. The data from the same experiment as used for section 1 above are plotted as line CPR in figure 3. As this plot shows, from 8 to 25 mm. Hg no flow was recorded. Above this pressure inflow occurred, increasing progressively as the perfusion pressure was raised. At perfusion pressures less than the animal's mean arterial pressure the inflow was greater and at pressures above mean arterial pressure the inflow was less than when the collateral arteries were perfused directly from the aorta. As indicated by the intersection of the CPA and CPR lines the flow into the cognate artery at a pressure equal to the animal's mean arterial pressure was

⁴ Liquaemin, containing 10 mgm. of heparin per milliliter. The solution was kindly supplied by Roche-Organon, Inc.

the same with the collateral arteries perfused directly from the aorta as when they were perfused from the reservoir at the same pressure as the cognate artery.

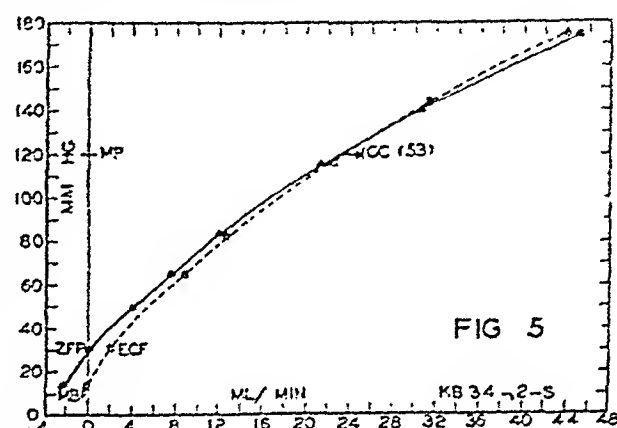
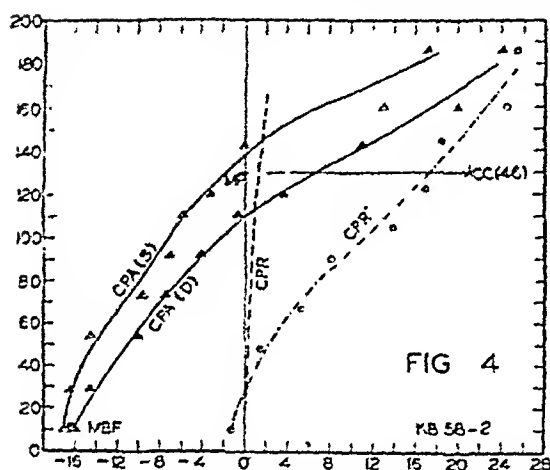
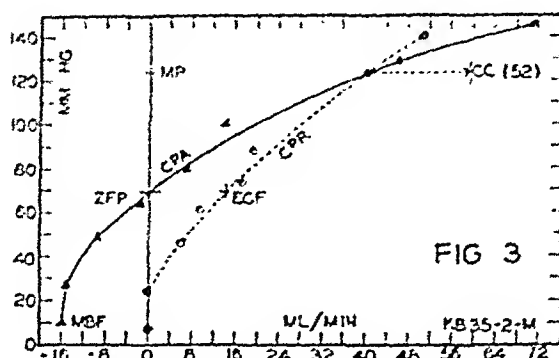


Fig. 3. Plots of the relationship of the perfusion pressure to the flow into the cognate arteries supplying the quadriceps muscle of the hind leg: *CPA*—solid line—relationship with the collateral arteries receiving blood directly from the aorta; *CPR*—dashed line—relationship with the collateral arteries receiving blood from the reservoir at the same pressure as the cognate artery. Ordinate scale—difference between perfusion pressure and peripheral venous pressure in millimeters of mercury. Abscissal scale—flow in milliliters per minute (—) figures represent backflow. *MP*—aortic mean pressure, *ZFP*—perfusion pressure at which neither inflow nor backflow occurred in the cognate artery when the collateral arteries were receiving blood directly from the aorta, *MBF*—maximum backflow, *ECF*—effective flow of blood through the cognate bed from the collateral bed which would occur immediately following occlusion of the cognate artery. *CC(52)*—rate of inflow into the cognate artery at a perfusion pressure equal to mean aortic pressure when the pressure in the collateral arteries was dropped to 52 mm. Hg.

Fig. 4. Plots similar to figure 3 for the tissues supplied by the popliteal artery—i.e., the portion of the femoral artery distal to the saphenous branch (an instance of very large potential exchange of blood between the collateral and the cognate beds). Lettering same as figure 3. For explanation of *CPA(S)*, *CPA(D)*, *CPR* and *CPR'*, see text.

Fig. 5. Plots similar to figure 3 for the tissue supplied by the saphenous artery branch of the femoral artery—almost solely skin (an instance of very small potential exchange of blood between the cognate and collateral vascular beds). For lettering see figure 3.

In view of the above observations, it appears to be a reasonable assumption that simultaneous perfusion of the cognate and collateral arteries at the same pres-

sure effectively prevents exchange of blood between the cognate and collateral beds. In other words, *under this mode of perfusion the rate of inflow in the cognate arteries is a true measure of the rate of flow through the cognate bed regardless of the perfusion pressure used.*

3. *The magnitude of the exchange of blood across the anastomotic channels at different perfusion pressures when the collateral arteries were perfused from the aorta.* The magnitude and direction of the exchange of blood across the anastomotic communications at any perfusion pressure applied to the cognate artery can be computed by subtracting the rate of flow on the CPA plot from that at the same pressure on the CPR plot. Thus, at a pressure 15 mm. Hg above mean aortic pressure blood flowed from cognate to collateral beds at the rate of 20 ml./min., indicating an exchange of blood from the cognate to the collateral bed equal to approximately 40 per cent of the flow through the cognate bed at this pressure. Similarly, at a pressure of 15 mm. Hg below mean aortic pressure blood flowed from the collateral to the cognate bed at a rate of 6 ml./min., equal to approximately 20 per cent of the flow through the cognate bed at this perfusion pressure.

B. *Blood flow into the popliteal artery and into the saphenous artery.* Blood flow into the popliteal artery was recorded in six experiments, and into the saphenous artery in ten experiments. In six of the latter the registration was made simultaneously with measurement of flow into the arteries supplying the vascular bed of the quadriceps muscle as illustrated in figure 2. Figure 4 reproduces typical results obtained from the vascular bed of the lower part of the hind leg supplied by the popliteal artery and figure 5 shows results from the vascular bed of the skin supplied by the saphenous branch of the femoral artery. The following should be noted: 1. The zero flow pressure (ZFP) is higher in figure 4 and lower in figure 5 than in figure 3. 2. In proportion to the inflow at mean aortic pressure the backflow is much greater in figure 4 and much less in figure 5 than in figure 3. 3. The exchange of blood between the cognate and collateral beds when the cognate artery was perfused at a pressure 15 mm. Hg greater or less than aortic mean pressure, while the collateral arteries were receiving blood from the aorta, amounted to the astonishing figure of 300 per cent of the flow through the cognate bed in figure 4, and only 3 per cent in figure 5.

In view of the high zero flow pressure (ZFP) and the high degree of exchange in figure 4, it is evident that the resistance to flow in the anastomotic channels must have been low in comparison with that in the cognate bed. Conversely, in figure 5 the resistance to flow in the anastomotic channels must have been high relative to that in the cognate bed. In the experiment plotted in figure 4 the resistance to flow in the anastomotic channels was, in fact, so low that a pulsatile flow was transmitted across the anastomotic channels when the collateral arteries were connected with the aorta. As a result, the zero flow pressure (ZFP), the inflow and the backflow all oscillated between maximum CPA (S) and minimum CPA (D) values with each heart beat.

C. *The effective collateral circulation immediately following occlusion of an artery.* The magnitude of the collateral blood supply available to a vascular

bed following sudden occlusion of its artery is often experimentally estimated by ligating the artery, cannulating the distal end and measuring the rate of backflow. In figure 3 this backflow (MBF) is 16 ml./min., in figure 4 it is 17 ml./min., and in figure 5 it is 2.2 ml./min. These are respectively 40, 1670 and 9 per cent of the normal flow through the cognate bed at mean aortic pressure.

The effective collateral circulation is, however, that rate of flow of blood which actually passes from the collateral beds across the anastomotic channels and *through the cognate bed* when the cognate artery is occluded. The magnitude of this effective collateral flow is given in the figures by the rate of flow on the CPR plot at the perfusion pressure corresponding to the zero flow pressure on the CPA plot. This is deduced from the facts, *a*, that the zero flow pressure (ZFP) on the CPA plots in the various figures is the pressure in the peripheral portion of the cognate artery when an effective collateral flow is occurring, and *b*, that the CPR plot gives the actual rate of flow through the cognate bed at each level of pressure in the peripheral portion of the cognate artery. The effective collateral circulation in figure 3 is 14.3 ml./min., in figure 4 it is 1.2 ml./min., and in figure 5 it is 2 ml./min. These flows are respectively 36, 85 and 9 per cent of the flow through the cognate bed at mean aortic pressure. In the hind extremity the least effective collateral circulation with respect to the normal flow is in the skin and the greatest in the muscles of the calf of the leg. The effective collateral circulation in the above experiments was respectively 90, 7 and 90 per cent of the maximum backflow. Evidently, measurement of backflow alone is not an entirely satisfactory method of estimating the effective collateral circulation, especially in relationship to the normal flow.

Peripheral arterial pressure (zero flow pressure—ZFP—as used above) has frequently been used as an estimate of the relationship of the effective collateral circulation to the normal flow, without definite proof of its significance (4, 5). The correctness of this assumption is now demonstrated by the above observations. Empirically, it appears from analysis of a number of experiments that a slightly better relationship is obtained by using the ratio of the peripheral arterial pressure minus 20 to the mean aortic pressure (6).

D. Effects of vasoconstriction upon the exchange of blood between the cognate and collateral vascular beds. In general, increased vasomotor tone diminished both the blood flow through the cognate vascular bed at each perfusion pressure and the exchange of blood by way of the anastomotic channels. The results, in terms of plots such as figures 3 to 5, are to make the CPA and CPR lines more nearly superimposed and steeper. Evidently, the anastomotic channels, as well as the arterioles of the cognate and collateral beds are supplied with muscle and are capable of undergoing vasoconstriction. A few exceptions were noted, however, in which the cognate bed and the anastomotic channels reacted independently.

E. Effect of changing aortic pressure upon flow into the cognate artery when the collateral arteries are perfused from the aorta. A common method of studying vasomotor reactions is to perfuse a vascular bed in situ with blood at a constant head of pressure. The interpretation of the results in such an experiment is

predicated on the assumption that all the blood which enters the cognate artery passes through its vascular bed and that conversely no blood reaches the cognate vascular bed from any other source. This condition is satisfied for most regions only so long as the head of pressure in the perfusion system is equal to the animal's mean aortic pressure. The magnitude of the possible artifact caused by exchange of blood between the cognate and collateral vascular beds due to a change of aortic pressure could be appreciated by inference from the discussion in section C. However, direct evidence was obtained in another manner. In each experiment, in addition to recording the rate of inflow at a perfusion pressure equal to mean arterial pressure with the collateral arteries perfused from the aorta, we also recorded the inflow at this same perfusion pressure when the pressure in the collateral arteries was dropped to whatever level it would reach as a result of compressing the aorta without simultaneously perfusing it below the clamp. In the experiment in figure 3 the pressure in the collateral artery fell to 52 mm. Hg following compression of the aorta and as a result the flow into the cognate artery increased 40 per cent—from 40 to 57.5 ml./min., as indicated by the horizontal arrow pointing to CC(52). Such a drop of pressure in the collateral arteries might just as well have occurred as a result of a decline in aortic pressure in a simple perfusion experiment in which the collateral arteries were perfused from the aorta. In the absence of knowledge regarding the presence of and the magnitude of the exchange of blood across the anastomotic channels in such an experiment the increased inflow would have been interpreted as due to a lowering of the peripheral resistance in the cognate bed, as indicated by the horizontal arrows pointing, respectively, to CC(46) and to CC(53) in figures 4 and 5, the increases of flow into the cognate artery as a result of a decline of pressure in the collateral arteries were respectively 1900 per cent and 8 per cent of the flow through the cognate bed. Similar studies of the effect of elevating the pressure in the collateral arteries, thus simulating a rise of aortic pressure, while perfusing the cognate arteries at a constant pressure were not carried out. It is reasonable, however, to assume from the data presented in section C that the error would be similar in magnitude to that just described for a fall of aortic pressure.

In some experiments reported a number of years ago, Erlanger, Gesell and Gasser (7) attempted to minimize the effects of this variable exchange of blood between the cognate collateral systems by perfusing the leg at pressures well above mean aortic pressure. However, data which we have obtained in these studies, but which we have omitted in order to simplify this presentation, indicate that the artifact due to changing aortic pressure may at high perfusion pressures be as large as or larger than at pressures nearer mean aortic pressure.

F. *Possible technical errors.* In the experiment plotted in figure 4 the dot dash line CPR' gives the actual rates of flow into the cognate artery when the collateral arteries were simultaneously perfused from the reservoir. Two artifacts indicate that this line does not give the correct data on the flow through the cognate bed: 1, backflow was observed at the lowest perfusion pressures, and 2, at a perfusion pressure equal to mean aortic pressure the inflow was much

greater than when the collateral arteries were being supplied by the aorta. The first of these indicates that blood was still able to enter the collateral circulation from the aorta at some point above the clamp and thus pass across the anastomotic channels to the cognate bed. The second was apparently due to the inability of the reservoir system to drive enough blood into the collateral systems to maintain a pressure in their distal branches equal to the pressure in the distal branches of the cognate artery. As a result, in the presence of the low resistance to flow in the anastomotic channels found in this experiment a large amount of blood flowed from the cognate to the collateral beds, thus causing the measured flow into the cognate artery to be much larger than the flow through the cognate bed. The correct plot would have been approximately that indicated by the dashed line CPR. This illustration presents the most extreme example in our experiments. These artifacts in less severe form were, however, seen in a number of experiments. The presence of either of these errors in experiments for studying the relation of perfusion pressure to flow through the cognate bed requires either that the experiment be discarded or that extreme care be used in deducing the correct relationship.

II. EFFECTS OF COLLATERAL CIRCULATION ON VENOUS OUTFLOW. A common practice in studying vasomotor reactions is to measure venous outflow when the vascular bed is perfused from the aorta. As long as there is only one vein draining the entire bed, this method is entirely satisfactory. However, if collateral venous paths are available, as indicated in figure 1, venous outflow measurements may fail to indicate correctly the flow through the cognate bed. This possibility was examined in the following set of experiments.

Methods. In four dogs, anesthetized with morphine and sodium barbital, combined arterial inflow and venous outflow measurements were made on the tissues supplied by the popliteal artery. In these experiments, venous outflow was recorded from cannula *COG VF* inserted into the saphenous vein as shown in figure 1, while the femoral vein was clamped centrally to the branch by *CS*. The tip of the outflow tube was placed at a level at which blood would just not flow out when the vein was not clamped, that is, at a level corresponding with the peripheral venous pressure. Mean arterial inflow was recorded with apparatus similar to that illustrated in figure 2. In these experiments blood flowed from the right femoral artery (*RFA*) through stopcock *SC 2*, orifice *O* and thence into a cannula inserted in the central stump of the saphenous branch of the left femoral artery while the latter was clamped centrally to the cannula. Reservoir *B* was left in the circuit as a pressure equalization chamber in order to damp the pulsations and obtain mean flow, which is more accurately measured than pulsatile flow.

Results. Simultaneous registration of the venous outflow and of the arterial inflow at mean aortic pressure was made at normal and at low aortic pressures produced by bleeding. These studies revealed that the venous outflows varied anywhere from 25 to 200 per cent of the arterial inflow. Since under this method of perfusion the arterial inflow may be expected to give a correct indication of the flow through the vascular bed such large discrepancies can be accounted for

only by assuming that an extremely small difference in pressure between the cognate and collateral veins can cause a very large exchange of blood across the venous anastomotic channels.

III. ELIMINATION OF EXCHANGE OF BLOOD BETWEEN COGNATE AND COLLATERAL BEDS BY MEANS OF WIRE TOURNIQUETS. Because of the extensive dissection required by the method described in section I in order to perfuse the collateral circulation adequately, we attempted to isolate the vascular bed of the hind extremity mechanically by applying two wire ligatures at about the junction of the middle and lower thirds of the thigh so as to compress all structures except the femoral artery and vein and the saphenous and sciatic nerves. This is comparable to compressing the anastomotic channels at the points shown by the arrows in figure 1. In order to check the adequacy of this method both arterial inflow and venous outflow were simultaneously recorded in two experiments. When the ligatures were insufficiently tight the arterial and venous flows were equal at perfusion pressures equal to aortic mean pressure, but at pressures above mean aortic pressure arterial inflow was greater than, and at pressures below mean aortic it was less than venous outflow. Furthermore, at very low perfusion pressures, backflow was obtained from the artery at the same time that outflow was recorded from the veins. Evidently, incomplete ligation blocked venous collateral channels but did not completely block arterial anastomotic channels. When the ligatures were adequately tightened, however, arterial inflow and venous outflow became identical. It is apparent, therefore, that, when properly applied, the ligatures eliminate both venous and arterial collateral circulatory artifacts.

SUMMARY

A *cognate* system is defined as a vascular bed, the blood flow characteristics of which are being studied, and its primary artery and vein. It includes only those arterioles, capillaries and veins which receive blood from the cognate artery when the latter is receiving blood from the aorta or from a reservoir at a pressure equal to aortic mean pressure while all collateral arteries are being supplied from the aorta. Collateral systems include all arteries, capillaries and veins which communicate with the cognate system by pre- and/or post-capillary anastomotic channels.

The magnitude of the exchange of blood across these anastomotic channels under various conditions was studied by measuring the flow of blood from a reservoir into the cognate artery at each of a series of pressures from zero to above mean aortic pressure, a , while the collateral arteries were receiving blood from the aorta, and b , while the collateral arteries were also being perfused from the reservoir at the same pressure as the collateral artery.

In experiments, on the hind extremity of the dog, in which the collateral arteries were receiving blood from the aorta, and in which the resistance to flow in the arterial anastomotic channels was small in relation to that in the cognate capillary bed it was observed that: a , flow into the cognate artery at a perfusion pressure 15 mm. Hg greater or less than the aortic mean pressure differed from

the flow through the cognate bed by 40 to 300 per cent; and *b*, although the flow through the cognate bed was unchanged the flow into the cognate artery under a constant head of pressure provided by a reservoir increased 40 to 1900 per cent as a result of fall in the pressure in the collateral arteries such as might be produced by a decline of aortic pressure.

The effective collateral flow is defined as the rate at which blood will flow from the collateral beds through the anastomotic channels and thence through the cognate bed immediately after occlusion of the cognate artery. Measurement of the backflow from the distal end of the cognate artery provides a rough measure of the effective collateral flow. A better estimate of the effective collateral flow with respect to the normal flow through the cognate bed at mean aortic pressure is given by the ratio of the peripheral arterial pressure in the cognate artery minus 20 to the mean aortic pressure. A still more accurate measure of the effective collateral flow is obtained by recording the flow into the cognate artery while perfusing the cognate and collateral arteries at a pressure equal to the peripheral arterial pressure (above) and comparing this with the flow through the cognate bed at mean aortic pressure. In various portions of the hind extremity of the dog the effective collateral flow varied from 9 to 85 per cent of normal flow through the cognate bed.

Studies of the relationship of perfusion pressure to flow through the cognate bed may be satisfactorily accomplished in most vascular regions by perfusing the collateral arteries at the same pressure as the cognate artery. However, technical errors are very likely to occur whenever the flow into the collateral arteries is extremely large or the anastomotic communications are very prominent.

In the presence of prominent venous anastomotic channels, outflow from the cognate vein may vary from 25 to 200 per cent of the flow through the cognate bed.

In flow studies in the distal part of extremities the exchange of blood across both venous and arterial anastomotic channels may be effectively prevented by application of wire ligatures which tightly compress all structures except the cognate artery and vein and the nerves.

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RED CELL VOLUME, PLASMA ALBUMIN AND GLOBULIN IN FATAL SURGICAL SHOCK DUE TO REPEATED HEMORRHAGE

ROBERT ELMAN, CARL LISCHER AND HARRIET W. DAVEY

From the Department of Surgery, Washington University Medical School and Barnes Hospital. Aided by a grant from the Commonwealth Fund

In previous communications (1, 2, 3) in this Journal, the behavior of the red cell volume, plasma protein including the albumin and globulin fractions was studied after a single severe non-fatal hemorrhage. Although a fall in blood pressure did occur and symptoms of surgical shock developed, compensation was adequate and no fatalities resulted. In order to study the effects of surgical shock ending fatally it was necessary to use a more severe type of hemorrhage. To do this, a standardized technic of repeated hemorrhage was adopted. The details have been described in a previous communication (4) in which the behavior of the blood pressure and the effect of various replacement fluids was described. In this communication changes of the red cell volume, serum albumin and globulin will be examined in detail with special reference to the influence of the length of the intervals between bleedings.

EXPERIMENTAL PROCEDURES. Mongrel dogs were used varying in weight between 9 and 18 kgm. They were prepared for the experiment by withholding all food for 18 hours. Water but no food was given during the course of the experiments. Each hemorrhage consisted of 10 cc. of whole blood per kilogram of body weight and was carried out by exposing the femoral artery, using local anesthesia when necessary, or by puncture of the artery without exposure thereof. No general anesthetic was used. Six groups of experiments were performed as follows:

GROUP	NUMBER OF EXPERIMENTS	INTERVAL BETWEEN BLEEDINGS
		hours
I	20	1
II	10	2
III	10	4
IV	10	8
V	10	12
VI	5	24

In groups I and II the animal was maintained in the same position during the entire procedure. In the other experiments it was placed in the cage between bleedings. In groups III, IV, V and VI bleedings were done by puncture of the femoral artery rather than exposure thereof in order to avoid the possibility of infection. This technic proved quite satisfactory and only rarely did extravasation of blood occur. The blood removed was not replaced with the exception of 10 of the experiments in group I in which the amount of blood removed was replaced with an equal volume of physiological saline solution. In group VI

three of the animals were allowed to eat ad libitum, whereas two were allowed water only.

EXPERIMENTAL FINDINGS. First in interest is the survival time in the various groups of experiments. Second is the behavior of the red cell volume which, as indicated in a previous communication, gives us a measure of the degree of hemodilution. Third are the changes in the albumin and globulin fractions which measure the comparative manner in which this diluting fluid replaces into the blood stream each of these two protein fractions. Data in regard to the blood pressure are not included in this communication although such observations were made. Hypotension occurred in all experiments, particularly just before the fatal outcome; details are described in a previous paper (4).

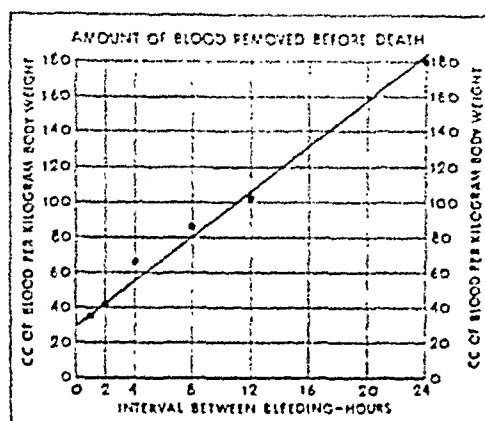


Fig. 1

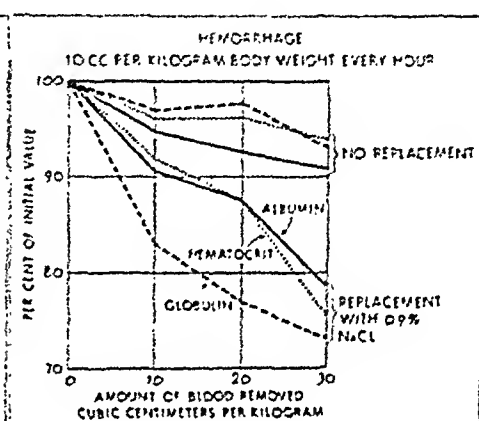


Fig. 2

Fig. 1. Note the progressive increase in the ability of the animal to withstand repeated hemorrhage as the interval increases between bleedings. With an interval of 24 hours note that the amount of blood which could be removed was nearly twice that normally present due obviously to compensation (regeneration).

Fig. 2. Note the pronounced hemodilution produced when the amount of blood removed was replaced with physiological sodium chloride solution. Note also that the drop in albumin was relatively less pronounced under these circumstances than in the experiments with no replacement.

Survival time. The survival time is presented in figure 1 in terms of the amount of blood which could be removed before death; this is plotted against the interval of time between bleedings. Inasmuch as the same amount of blood was removed at each bleeding, this manner of presentation actually represents survival time. It will be seen that the graph forms a straight line and indicates rather clearly that the survival following repeated hemorrhage bears a direct relationship to the interval between bleedings. In other words, the compensatory mechanisms of the body following repeated hemorrhage are much more effective in prolonging life the longer the time allowed for such compensation. Indeed, if 24 hours elapsed between each hemorrhage, as many as 17 bleedings occur without a fatality, i.e., nearly twice as much blood can be removed as is normally present. Although only 5 experiments were carried out in this group, it was of interest to note that the 3 animals which were fed ad libitum survived

this ordeal as well as those who were given only water by mouth. Loss of weight was quite pronounced in all of these 5 animals and the hematocrit dropped to tremendously low values.

The effect of replacement. In group I half of the animals were treated by replacing the amount of blood removed with an equal volume of physiological saline. It will be noted in text figure 2 that this replacement resulted in a progressively greater fall in the red cell volume as well as the albumin and globulin concentrations. However, the survival time was no different with replacement than without replacement. These findings are to be compared with previous experiments in which other solutions were used for replacement in a similar series of experiments.

Variations in cell volume, albumin and globulin. In text figure 3 are represented the variations in red cell volume, albumin and globulin concentrations in

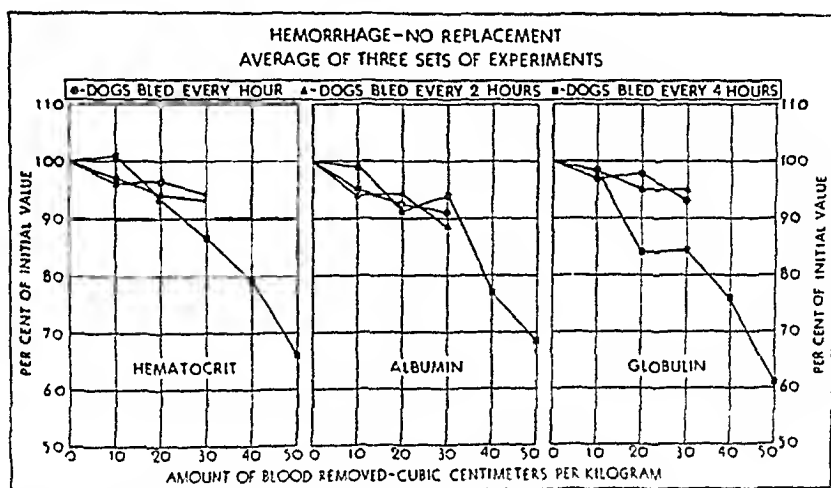


Fig. 3. Note the similarity in the data between the one and two hour hemorrhages. Note also that with the four hour hemorrhages the hemodilution was increased, indicating that time is necessary for the mobilization of interstitial fluids. Of special interest is the fact that the albumin fraction dropped much less than the globulin fraction in these latter experiments.

bleeding experiments performed at intervals of 1, 2 and 4 hours. These findings give some indication of the spontaneous compensatory mechanisms regarding hemodilution and the return of albumin and globulin in relationship to time. In general it will be noted that there is very little difference between the bleeding experiments at intervals of one hour and those of 2 hours. However, there is a striking difference in the experiments in which the blood was removed at intervals of 4 hours. In addition to the fact that this latter group survived a much larger amount of bleeding, the hemodilution after the second bleeding was much more pronounced and was progressive. Moreover, the albumin concentration was maintained better than in the other experiments, at least for the first 4 bleedings. After this point, its concentration fell rather rapidly even though the degree of hemodilution was unchanged. This would seem to indicate that albumin was added to the diluting fluid up to and including the fourth bleeding,

but thereafter its source probably was exhausted. This behavior of the albumin fraction is to be contrasted with the behavior of the globulin fraction which fell progressively after the second bleeding, thus indicating that its source was exhausted much earlier than in the case of albumin.

COMMENT. Study of the experimental findings described above shows that the compensatory mechanisms following repeated hemorrhage require time and that the beneficial effect of time is progressive and directly proportional to the interval between hemorrhages. There seems to be little difference between one and two hours, but after this period the ability of the body to compensate for the effects of loss of blood is significant. Indeed, when an interval of 24 hours elapses, compensation is so good that a loss of nearly twice the normal blood volume can be sustained without fatality.

Further study of the present findings furnishes inferences in regard to the mechanism of this compensation. In the absence of any replacement of fluid it is apparent that a period longer than 2 hours is required for significant hemodilution, i.e., the mobilization of fluids requires at least 2 hours before it becomes of significance. This finding agrees rather well with previous studies (2) on single hemorrhages, in which hemodilution was shown to proceed rapidly for about 6 hours after hemorrhage, continuing much more slowly up to 72 hours. However, hemodilution in itself (i.e., with protein free fluid) is of little significance in compensation, as illustrated by the findings shown in text figure 2, in which the survival time was not altered even though pronounced hemodilution was induced by replacement of physiological saline solution.

Hemodilution, to be effective in compensating for the loss of blood, must contain protein, in particular albumin, and study of these two fractions shows that time is also necessary for its mobilization and entrance into the blood stream. As shown in text figure 3 bleedings at intervals of 4 hours permit entrance of albumin for at least 4 bleedings in contrast to globulin which apparently has no such rapidly available source. These considerations are important when considering the phenomenon of surgical shock from hemorrhage based on a biochemical rather than a physiological or physical point of view. Such an approach suggests that the fatal outcome following hemorrhage is in reality due to an acute hypoalbuminemia, the albumin fraction being of greater significance than the globulin fraction because of the fact that the former contributes more to the colloidal osmotic pressure than the latter.

The relatively slight fall in the red cell volume of the experiments in group 1 in which there was no replacement (upper curve in fig. 2) deserves comment. Ordinarily a single severe hemorrhage is followed by a great fall in hematocrit even when death supervenes. It would seem that in the present experiments with repeated hemorrhage each hour this fall is inhibited. A possible explanation is the finding that under these circumstances the fall in blood pressure is relatively slight (except for the terminal fall) as described in a previous paper. Hemodilution as shown by a fall in the hematocrit probably depends, according to Starling's hypothesis, upon a fall in capillary pressure, and if this does not occur because of a relatively high systemic blood pressure, hemodilution may be inhibited.

SUMMARY

Fatal surgical shock followed repeated hemorrhage, each bleeding amounting to 10 cc. per kilogram of body weight. When the interval between bleedings was one or two hours, but 30 to 40 cc. could be thus removed. When the interval was increased, relative survival was prolonged as shown by the fact that the amount which could be removed before death increased in simple arithmetical progression. Study of red cell volume changes shows that at least 2 hours between bleedings are required for significant hemodilution and that when this interval is increased there is evidence of the addition of albumin to the diluting fluid, in contrast to globulin which was not restored in any of the experiments. Artificial hemodilution by replacing the blood with physiological saline did not prolong life. These findings are described in support of the idea that acute hypoalbuminemia is an important cause of failure to compensate for the loss of blood.

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